Monograph on **Dextrans** #111 2/14/74

DEXTRANS #111

#111

informatics inc

M O N O G R A P H

O N

D E X T R A N S

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DEXTRANS

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DEXTRANS

Summary

Despite the use of dextrans as food additives, very little information is available on the effects of orally administered dextran. Several workers have shown that extracts of various tissues of different mammals, including man, possess dextranase activity, the activity being localized primarily in the small-intestinal mucosa (14, 100, 195, 196). Bloom and Wilhelmi (100) have shown that in both rat and man the oral administration of dextran leads to a significant and sustained increase in blood reducing substance and in liver glycogen. Parkinson (772) also observed an increase in liver glycogen of rats following dextran feeding. Baker stated, without supporting data, that while dextran is edible and assimilated without unfavorable effect on the human system, it appears that the alpha-(1-6) linkages are resistant to attack by bacteria and enzymes present in the gastro-intestinal tract (54). He also stated that biological tests (unspecified test animals) have demonstrated that when dextran containing a high proportion of alpha-(1-6) linkages is included in a normal diet on a regular regimen, gain in body weight is inhibited (54).

A good deal more information is available on the effect of parenterally administered dextran. During the World War II period dextran was considered for possible extensive use as a plasma expander. As a result, a great deal of research was conducted on the effects of intravenously administered dextran. An excellent review of this work was compiled by Squire et al. (950) in 1955. Rather than try to summarize the work of Squire et al., which is of questionable value in the evaluation of a food additive, their review is reproduced in its entirety in the back-up material of this monograph.

In an extensive study by Hueper (461), eleven different dextrans were administered to mice, rats and rabbits in single and multiple doses through s.c., i.p., and i.v. routes. The maximal observation period for the mice and rats was two years; for the rabbits, up to four years (461). On the basis of his results, Hueper (461) concluded that some, but not all, dextrans elicit upon parenteral introduction into rats and mice, and perhaps also in rabbits, sarcomas originating from organs and tissues in which the substance is retained and stored, i.e., the reticuloendothelial tissues. In a study by Lusky and Nelson (647), 10 male and 10 female Osborne-Mendel rats and 10 male Bethesda Black rats were given weekly s.c. injections of 1 ml of 6% dextran for 73 weeks without the formation of any injection-site tumors. Richmond (830) reported that a highly significant number of rats given i.m. injections of an iron-dextran complex developed injection-site tumors while rats given dextran alone developed no injection-site tumors.

Numerous studies, many of which appear to contradict each other, have been conducted on the effects of intravenously administered dextran on tumor promotion in animals inoculated with tumor cells. Typical of the studies in which dextran was found to promote tumor growth, Hagmar (393) and Fisher and Fisher (281, 282) found that i.v. injection of dextran enhances the production of tumors in mice, rats and rabbits treated with tumor cell suspensions. On the other hand, Wood et al. (1094) reported that i.v. administration of dextran to rabbits before or after inoculation

with carcinoma cells failed to significantly alter the incidence of tumor formation. Further studies of this sort were excluded from this monograph.

DEXTRANS

Chemical Information

I. Nomenclature

A. Common Names

1. Dextran

B. Chemical Names

1. Dextran

C. Trade Names

1. Macrose
2. Macrodex
3. Intradex
4. Polyglucin
5. Dextraven
6. Onkotin
7. Expandex
8. Gentran
9. Plavolex

C. Chemical Abstracts Registry Number

PM9004-54-0

II. Empirical Formula

$(C_6H_{10}O_5)_n$

III. Structural Formula

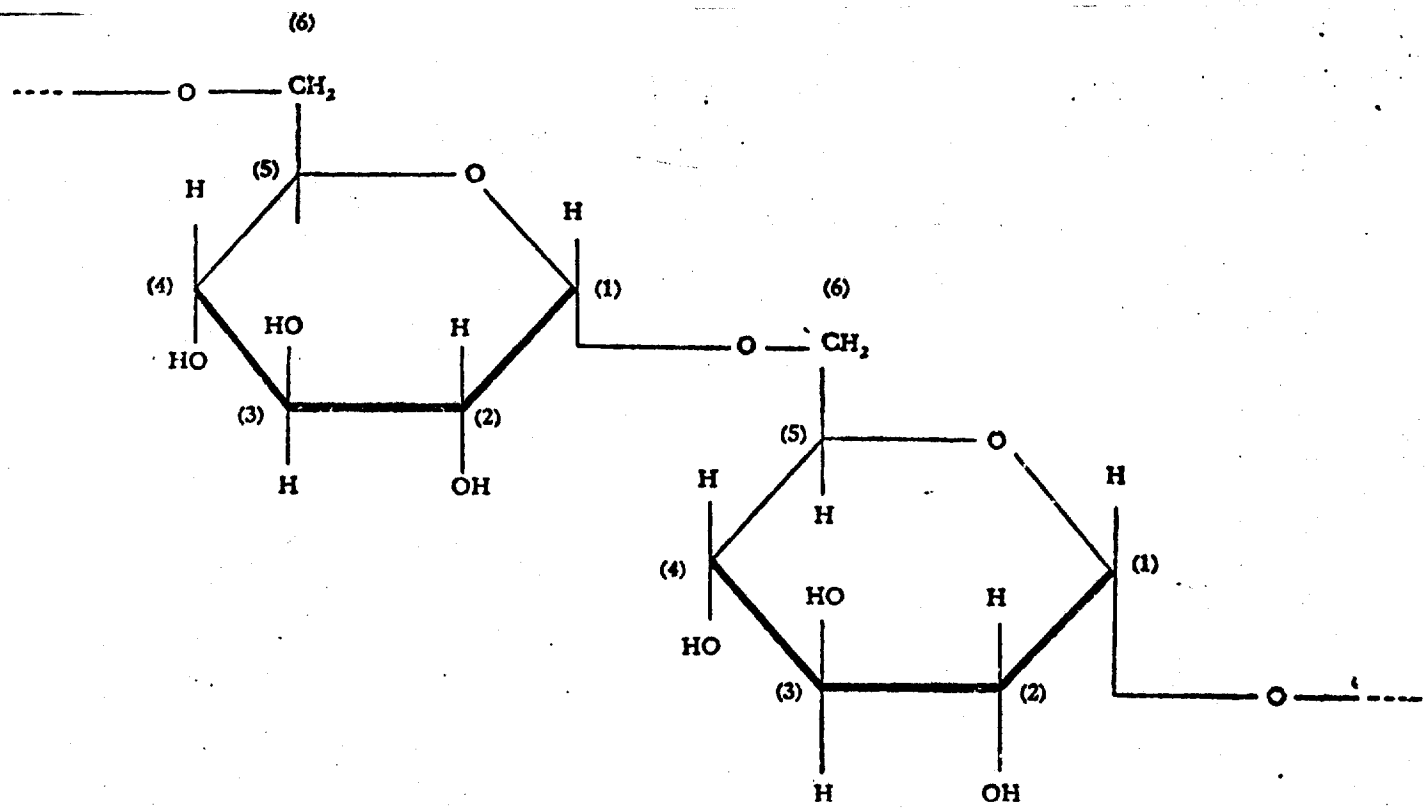

FIG. 2. Two glucose units forming part of the molecular chain in dextran. Dextran is characterized by the alpha 1 : 6 link between the two units.

IV. Molecular Weight

Dextran can vary widely in molecular weight (values of up to several million have been reported), and any dextran can be degraded to dextrans having essentially the same structure but of considerably lower molecular weights.

V. Specifications

A. Chemical

No Information Available

B. Food Grade

No Information Available

C. Clinical

The specifications for dextran to be used as a plasma expander are as follows (950):

CHEMICAL AND PHYSICAL SPECIFICATIONS FOR CLINICAL DEXTRAN

U.S.A. (Military Medical Purchase Description, 1954)	*Great Britain* (Ministry of Health Specification, 1951)
Analysis:	*Analysis*:
Dextran 5.7–6.3 g./100 ml.	Dextran 5.5–6.5 g./100 ml.
Sodium chloride 0.85–0.95 g./100 ml.	Sodium chloride 0.85–0.95 g./100 ml.
Buffering capacity ≯ 3.0 ml. 0.1 N NaOH/litre	Potassium ≯ 25 mg./100 ml.
pH 4.5–7.0	pH 5.0–7.0
Nitrogen < 1.00 mg./100 ml.	Reducing sugars < 100 mg./100 ml.
Heavy metals as lead < 0.5 mg./100 ml.	Acetone < 0.02 g./100 ml.
Ash (less NaCl) < 0.05 g./100 ml.	Nitrogen < 1.00 mg./100 ml.
	Heavy metals as lead < 0.5 mg./100 ml.
Molecular Composition:	*Molecular Composition*:
Whole polymer M_W 75,000 ± 15,000	Intrinsic viscosity 0.32 ± 0.05 dl./g. in aqueous solution at 37°C.
High 10% fraction M_W ≯ 200,000	High 10% fraction—intrinsic viscosity ≯ 0.53 dl./g. in aqueous solution at 37°C.
Low 10% fraction M_W ≮ 25,000	Renal excretion (under stated conditions in rabbits) < 25%
Inherent viscosity 0.255 ±0.035 dl./g. in aqueous solution at 25°C.	

VI. Description

A. General Characteristics

See Next Section

B. Physical Properties

Jeanes et al. characterized the dextrans produced by 96 individual bacterial strains. The properties of these dextrans are presented in Table 1 (501).

TABLE I

PROPERTIES AND CLASSIFICATION OF PURIFIED DEXTRANS FROM 96 DIFFERENT STRAINS OF BACTERIA. IDENTITY AND ORIGIN OF THE STRAINS

Strain no. NRRL B-	Type AGU links, % 1,6-	1,4-like	1,3-like	Dextran [α]²⁵D (c 1) HCONH₂	1 N KOH	Dextran Viscosity, water, 25° [η]	k_1	Yield, %[a]	Solubility, water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
Class A Dextrans. 0–2% 1,3-like links														
1146	97	3	0	+214		1.245	1.07	11	+	Long	L.d.	NCIB	3356	P-2615[17]
1064	96	4	0	214		0.887	0.91	29	+ p	Tough, stringy	L.m.	CSMc	548	Type D[18]
1414	96	4	0	214		.869	.96	15	+	Short	L.m.	Isolate		
1145	96	2	2	214		1.029	.83	24	+	Long	L.d.	NCIB	3355	52[17]
512(F)[27]	95	5	0	215	+203	0.953	1.10	24	+	Long	L.m.	RGB[14]		Substrain of B-512[27]
640	95	5	0	214		1.280	1.03	14	+	Long	L.d.	ATCC	8086	22[17]; its dextran[7]
1066	95	5	0	215		0.521	0.83	11	+ p	Crumbly, F[g]	L.m.	CSMc		Subtype of type D[18]
1208	95	5	0	213		.628	1.37	17	+ p	Crumbly	L.m.	CSMc		Type D[18]
1210	95	5	0			.698	1.33	24	+	Short, rough	L.m.	CSMc		Type D[18]
1211	95	5	0	214		.843	1.30	16	+	Short, smooth	L.m.	CSMc		Type D[18]
1308	95	5	0	219		.476	1.37	15	+ p	Pasty, crumbly, F	L.m.	BJH		
1209	95	3	2	215		.693	0.87	18	+	Short, smooth	L.m.	CSMc		Type D[18]
1119	94	4	2	217		1.617	.86	7	+	Cohesive, stringy	L.m.	ATCC	8357	
1072	94	6	0	216		0.883	.93	24	+	Long	L.m.	ARS		Substrain of B-512
1198	94	6	0	215		.760	1.39	23	+	Short, F	L.m.	CSMc		Type D[18]
1212	94	6	0					16	+	Short	L.m.	CSMc		Type D[18]
1380	94	6	0	215		.848	1.39	4	+	Short	u	ALP		Similar to strain reported[28]
-I	93	7	0			.968	1.17	2	+	Short, tough				
1405	94	6	0	216		.660	1.34	9	+	Short	L.m.	Isolate		
1412	94	6	0	216		1.127	0.98	12	+	Long	L.m.	Isolate		
1413	94	6	0			0.704	1.21	15	+	Short	L.m.	Isolate		
1417	94	6	0	217		.654	1.14	17	+	Short, F	L.m.	Isolate		
1412	94	6	0	214		1.019	0.86	16	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1204	93	7	0			0.846	1.28	18	+	Crumbly	L.m.	CSMc		Type D[18]
1214	93	7	0					21	+	Short, F	L.m.	CSMc		Type D[18]
1197	92	6	2	212		.510	1.13	8	+	Floc. ppt.	L.m.	CSP	683	9[17]
1307	91	9	0	215		.952	1.08	19	+ p	Short, tough	L.m.	JMN, BJH	"B"	References 10, 29, 30
1388	91	9	0			.917	1.09	16	+	Short, tough	L.m.	RP		
1225	90	10	0	208				24	+ p	Short	A.c.	BJH		NCTC 4043, Ref. (19)
1226	90	10	0	212		.704	0.87	20	+ p	Short	A.v.	BJH		NCTC 7216, Ref. (19)
1500	90	10	0	215	204	.823	1.71	19	+ p	Short, tough	(L.m.)	CSMc		Type F[18]
1415	89	11	0	216		1.180	0.91	12	+	Stringy	L.m.	Isolate		
1196	88	10	2	215		0.890	1.22	26	+	Short	L.m.	WWC	"elal"	Ref. (31)
1400	86	14	0			.950	1.02	7	+	Stringy	L.m.	Isolate		
1383	84	16	0	217		.957	1.12	15	+ p	Short, rough	L.m.	RP		
1410	84	16	0	216		.875	0.89	17	+	Short	L.m.	Isolate		
1525	83	17	0	217		.843	0.88	24	+	Fluid, stringy	L.m.	Isolate		
1390	82	18	0	216		.857	1.03	14	+ p	Short, stiff	L.m.	RP		
1382	81	19	0	218		.810	1.00	13	+ p	Short	L.m.	RP		

TABLE I (*Continued*)

Strain no. NRRL B-	Type AGU links % 1,6-	1,4-like	1,3-like	[α]²⁵D (c 1) HCONH₂	1 N KOH	Dextran Viscosity, water, 25° [η]	k_1	Yield, %[a]	Solubility water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
1536	81	19	0	217				19	+	Short, tough	L.m.	Isolate		
1420	81	19	0	216		.522	0.68	8	+	Short	L.d.	Isolate		
-I	80	20	0	214		.453	1.23	7	+	Short				
1526	79	21	0	216		.378	0.65	4	+		S.sp.	Isolate		
-I	77	23	0			.225	1.42	8	+	Short				
1397	75	25	0	219				21	+	Short	L.m.	Isolate		
1422	74	26	0	218		1.027	0.89	19	+	Short	L.m.	ERW		
1424	72	28	0	219		1.088	.75	17	+	Stringy	(L.m.)	JW	"D"	Refined Syrups and Sugars, Inc., strain "D" or 1053; derived from ATCC 6025
1402	66	34	0	220		0.925	.78	21	+	Short, F	L.m.	ERW		
1399	65	35	0	217		.913	.84	19	+	Short	L.m.	Isolate		
1298	64	36	0	223		1.025	.90	12	+ p	Short	L.m.	JMN	7 or "C"	Serol. type A[28,29]
						Class B Dextrans. 3–6% 1,3-like links								
1193	95	2	3	+218		0.578	1.34	5	+	Short	L.d.	CSP	853	
641	94	3	3	215		1.041	1.02	17	+	Long	L.m.	ATCC	8082	
1205	94	3	3			0.865	1.22	26	+	Short, F		CSMc		Type D[28]
1397	94	3	3	217		1.418	1.04	14	+	Short	L.m.	RP		
1407	94	3	3	216		0.572	1.73	13	+	Short, cohesive	L.m.	Isolate		
1419	94	3	3	217		.815	1.45	19	+	Short, tough	L.m.	Isolate		
1400	93	3	4	220		.795	0.87	5	+	Short	L.m.	Isolate		
1401	93	3	4	215		.446	.87	8	+	Short	L.m.	Isolate		
1394	92	4	4	215		2.020	.70	6	+	Cohesive, stringy	L.m.	FWF[32]		
-I	92	5	3			1.472	1.44	1	+					
1410	91	5	4	217				7	+	Short	L.m.	Isolate		
1392	91	6	3	218		0.555	0.96	9	+	Stringy	u	Isolate		
1255	89	7	4	219		.696	1.22	18	+ p	Floc. ppt., crumbly	S.d.	AJK	L-337	Isolation[33]
1127	89	5	6	220		.945	0.87	14	+ p	Long	B.v.	AJK	L-343	May be same as previously reptd.[5]
1502	87	8	5		208	1.043	.97	7	+ p	Short	(L.m.)	CSMc		Type F[28]
1144	87	7	6		209	1.153	.72	9	+ 120°	Short, tough	L.m.	NCIB	3354	
						Class C Dextrans. >6% 1,3-like links								
1120	85	0	15					9	−	Crumbly	L.m.	ATCC	8358	Type I[24]
1351	85	4	11	217		0.505	0.52	27	+	Short	S.v.	EJH		Ref. (35)
1389	85	7	8	220		1.102	1.23	21	+	Short	L.m.	RP		
1429	83	5	10		210	1.360	1.16	8	+ p	Crumbly	L.m.	CSMc		Type B[28]
1377	84	7	9	219		1.364	0.81	20	+	Long	L.m.	Svenska Sockerfabriks	AB VII-E	
1384	84	6	10	221				20	+ p	Tough	L.m.	RP		
1139	83	5	12		213	0.503	1.25	9	+ p 120°	Floc. ppt.	B.v.	AJK	L-344	Isolation[33]
1411	82	8	10	217		1.093	1.07	21	+	Short, tough	L.m.	Isolate		
1385	81	9	10	222	213	0.995	1.22	21	+ p	Crumbly	L.m.	RP		
1374	81	7	12	220		1.338	0.79	25	+	Stringy	L.m.	Benger's Ltd.		
1375	81	6	13	220		0.918	1.00	14	+	Short	L.d.	Dextran Ltd.		"Birmingham" strain[14,28]
1438	81	6	13		213	1.509[A]	0.89	4	+ 120°	Floc. ppt.	L.m.	CSMc		Type B[28]
1438-A	79	7	14		213	1.458[A]	.86	9	+ 120	Floc. ppt.				

TABLE I (*Continued*)

Strain no. NRRL B-	Type AGU links % 1,6-	1,4-like	1,3-like	$[\alpha]^{28}D$ (c 1) HCONH₂	NKOH	Dextran Viscosity, water, 25° $[\eta]$	k_1	Yield, %[a]	Solubility water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
1439	81	6	13	221		0.475	1.08	10	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1445	80	10	10	220		.418	0.86	18	+	Pasty	(L.m.)	CSMc		Type A[18]
1141	79	3	18	224		1.350	1.04	17	+	Tough, stringy	L.d.	NCIB	2706	63[17]
1192	78	4	18	223	210	0.910	1.33	22	+	Short, crumbly	L.m.	CSP	851	
1191	77	9	14	223		.882	1.35	19	+	Short, crumbly	L.m.	CSP	845	
1118	76	3	21		215	1.821[h]	0.74	9	−	Floc. ppt.	L.m.	ATCC	8293	
1425	74	8	18	222		1.105	.93	7	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1398	70	11	19	222		0.865	.91	19	+	Short	L.m.	Isolate		
1297	67	24	9	219	211			2	+ p, 120°	Short, rough	L.m.	JMN	5 or "A"	Ref. (10, 29, 30)
F90A[27]	67	2	31		225				+ p, 120	Floc. ppt.	S.v.	EJH		Lancefield group H[27]
523	66	10	24		220	2.081[h]	1.51	6	−	Floc. ppt.	L.m.	C. Thom	535	
1121	65	2	33		222			7	−	Floc. ppt.	L.m.	ATCC	8359	Type II[34]
1142	63	8	29	230		0.389	1.60	6	+ p	Floc. ppt.	L.m.	NCIB	3351	Same origin as NRRL B-742
1433	63	30	7		217	2.605[h]	1.17	17	−	Crumbly	L.m.	CSMc		Type B[18]
1433-A	63	30	7		217	2.514[h]	1.22	6	−	Tough				
1431	62	29	9		217	3.107[h]	0.34	10	−	Floc. ppt.	L.m.	CSMc		Type B[18]
1149	52	8	40		232	2.716[h]	1.24	4	−	Fine ppt.	L.m.	NCIB	6109	
Structurally heterogeneous dextrans and/or their major components														
742	67	21	12	+223		0.296	1.35	15.0	+ p	Short, dense	L.m.	CSP[18]	681	5[17]; 4[4]
-L	81	19	0	212		.152	1.38	(35)[g]	+ p	Fine ppt.				
-S	57	17	26	226		.326	1.45	(39)	+ p	Fine ppt.				
1254	90	7	3	216		.488	1.39	12	+ p	Floc. ppt.	S.d.	AJK	L-336	Isolation[32]
-L	69	31	0	213		.189	1.29	(7)	+ p	Floc. ppt.				
-S	93	7	0	214		.537	1.26	(55)	+ p	Floc. ppt.				
1299-L	58	36	6		+216	.873	1.05	(55)	+ p 120°	Floc. ppt.	L.m.	JMN 8 or "K"		From AJK, 1940. Serol. type A[27,33]
-S	50	50	0	221	212	.469	1.53	(23)	+	Fine ppt.				
1355-L	88	9	3		206	1.115	1.13	(37)	−	Short	L.m.	RP		
-S	57	8	35	233	220	0.193	1.24	(48)	+	Fine ppt.				
1498-A	91	9	0	212		1.156	1.81	5	+ p	Short, tough	L.m.	CSMc		Type F[18]
-L	94	6	0	213		1.096	1.30	14	+	Short				
-S	62	11	27	227		0.329	1.25	3	+	Fine ppt.				
1501-A	80	18	2	211		1.004	1.67	7	+ p	Short, dense	L.m.	CSMc		Type F[18]
-L	93	7	0		206	1.054	1.34	7	+	Short				
-S	65	15	20		216	0.412	1.28	5	+	Fine ppt.				

[a] Based on weight of sucrose in culture. [b] +, soluble; −, insoluble; p, if precautions are observed; 120°, solution completed by autoclaving. [c] Observed when precipitated from aqueous solution by ethanol of 45–50% concentration. Products are gums unless otherwise stated. [d] Identities are as confirmed or determined[34] except for those indicated in parentheses, which are as received. A, *Acetobacter;* B.v., *Betabacterium vermiforme;* c, *capsulatum;* d, *dextranicum;* L, *Leuconostoc;* m, *mesenteroides;* S.d., *Streptobacterium dextranicum;* S.sp., *Streptococcus* species; S.v., *Streptococcus viridans;* v, *viscosum;* u, unidentified. [e] NCIB, National Collection of Industrial Bacteria; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures. [f] Initials stand for names of donors, as follows: R. G. Benedict; W. W. Carlson; E. J. Hehre; G. J. Hucker; A. J. Khayyer; C. S. McCleskey; J. M. Neill; R. Patrick; C. S. Pederson; A. L. Pollard; A. R. Stanley; J. Warren and E. R. Wolford. [g] F, fluorescent in ordinary light as previously reported[31,32]. [h] Solvent, 1 *N* potassium hydroxide. [i] Values in parentheses are per cent. of the purified fraction obtained from the whole dextran.[18]

C. Stability

Upon examination of two dextran solutions which had been stored for 5 years at 4 degrees C, Maycock and Ricketts concluded that during the 5-year period there was little, if any, change in the molecular composition of the dextran solutions and none that would be noticeable in clinical use (684).

VII. Analytical Methods

The quantitative determination of the concentration of dextran in a solution can be carried out through total acid hydrolysis of the dextran to D-glucose and subsequent determination of the glucose by any standard procedure (54, 950).

VIII. Occurrence

Dextran is produced by the action of certain strains of chain forming microorganisms on sucrose.

Biological Data

I. Acute Toxicity

No Information Available

II. Short-Term Studies

No Information Available

III. Long-Term Studies

No Information Available

IV. Special Studies

Cancer

In 1959, Hueper published the results of an extensive study on the carcinogenicity of dextran. Eleven different dextrans were administered to mice, rats and rabbits in single and multiple doses through subcutaneous, intraperitoneal, and intravenous routes. The maximal observation period for the mice and rats was two years; for the rabbits, up to four years. At the conclusion of the observation period all survivors were sacrificed and autopsied. Histologic examinations of the tissues were made on all animals exhibiting grossly demonstrable pathologic changes or on at least 30% of animals of each series regardless of the presence or absence of such lesions. The organs of all rabbits were studied histologically. Normal untreated animals of the same strain and animals given parenteral implants or injections of various noncarcinogenic vehicles (wool fat, gelatin, tricaprylin) or test chemicals served as controls. They were permitted to live for the same length of time as the animals of the experimental series and were used for determining the type, age, and site distribution of spontaneous tumors (461).

The results of the studies are detailed in Table 1 (461).

Upon histological examination, the lungs of the rabbits frequently revealed intracapillary giant-cell granulomas, which were sometimes rather cellular and at other times mainly hyaline. Similar but much larger formations occasionally blocked the lumens of large pulmonary arteries. In the presence as well as in the absence of such foreign-body granulomas the endothelial lining of arteries exhibited sometimes focal increases of cells which at times produced small crescent-shaped, cellular plaques of the intima. Apparently older lesions of this derivation appeared as fibrous intimal thickenings (461).

Foam-cell accumulations in intrahepatic sinusoids were only occasionally observed in the livers of mice. In the spleen of one rabbit hyaline masses surrounded atrophic lymph follicles. The same rabbit exhibited also an extensive hyalinosis of the renal glomeruli (461).

Table 1

	Animal	Strain or type	Sex	Preparation and dose	Site and route	Animals with tumors	Survival	Duration of experiment
Hueper, W. C., 1959	20 rats	Bethesda Black		500 mg. powder once.[8370]	I.P.	Tumors[8371, 8378]	All survivors sacrificed.	2 yrs.
	[illegible]	Dutch		1,750 mg., 7% aqueous soln., total dose 17,500–35,595 mg.[8372]	I.V.	0	All survivors sacrificed.	Up to 4 yrs.
	40 mice	C57BL		200 mg. powder once.[8373]	S.C.	Tumors[8374, 8376]	All survivors sacrificed.	2 yrs.
	20 mice	C57BL		200 mg. powder once.[8375]	S.C.	Tumors[8375, 8377]	All survivors sacrificed.	2 yrs.

[8368] Predominately fibrosarcomas.
[8369] Fibrosarcoma.
[8370] 1 malignant tumor.
[8371] 3 malignant tumors.
[8372] Dextran 1; avg. molecular wt.: 200,000.
[8373] 1 nodal reticulum cell sarcoma, 2 mammary adenofibroma, 1 uterine carcinoma.
[8374] 240 controls with 11 reticulum cell sarcomas, 1 pulmonary adenoma, 3 mammary adenofibromas, 1 renal carcinoma, 4 uterine carcinomas, 1 ovarian carcinoma. Controls received various treatments. Survival rates not reported.
[8375] Dextran 2; avg. molecular wt.: 100,000.
[8376] 1 hepatic reticulum cell sarcoma.
[8377] 840 controls with 6 reticulum cell sarcomas. Controls received various treatment. Survivor rates not reported.
[8378] Dextran 4; avg. molecular wt.: 75,000.
[8379] 1 nodal reticulum cell sarcoma.

Table 1 cont.

Animal	Strain or type	Sex	Preparation and dose	Site and route	Animals with tumors	Survival	Duration of experiment
35 mice	C57BL		200 mg. powder once[5378]	S.C	Tumors[5376, 5378]	All survivors sacrificed.	2 yrs.
3 rabbits	Dutch		1,750 mg., 7% aqueous soln., total dose 17,500–35,500 mg.[5373]	I.V	0	All survivors sacrificed.	Up to 4 yrs.
20 rats	Bethesda Black		500 mg. powder once.[5373]	I.P	Tumors[5380, 5381]	All survivors sacrificed.	2 yrs.
20 rats	Bethesda Black		500 mg. powder once.[5379]	S.C	Tumors[5381, 5383]	All survivors sacrificed.	2 yrs.
20 mice	C57BL		200 mg. powder once.[5378]	I.P	Tumors[5376, 5382]	All survivors sacrificed.	2 yrs.
28 mice	C57BL		200 mg. powder once.[5384]	S.C	Tumors[5379, 5386]	All survivors sacrificed.	2 yrs.
13 mice	C57BL		200 mg. powder once.[5380]	S.C	0[5376]	All survivors sacrificed.	2 yrs.
9 mice	C57BL		[illegible]	S.C	Tumors[5376, 5377]	All survivors sacrificed.	2 yrs.
20 mice	C57BL		200 mg. powder once.[5380]	I.P	Tumors[5380, 5376]	All survivors sacrificed.	2 yrs.
40 mice	C57BL		200 mg. powder once.[5384]	S.C	Tumors[5380, 5375]	All survivors sacrificed.	2 yrs.
20 rats	Bethesda Black		500 mg. powder once.[5380]	S.C	Tumors[5381, 5380]	All survivors sacrificed.	2 yrs.
40 mice	C57BL		200 mg. powder once.[5379]	S.C	Tumors[5376, 5381]	All survivors sacrificed.	2 yrs.
20 mice	C57BL		200 mg. powder once.[5379]	I.P	Tumors[5380, 5376]	All survivors sacrificed.	2 yrs.
20 rats	Bethesda Black		500 mg. powder once.[5379]	S.C	Tumors[5381, 5380]	All survivors sacrificed.	2 yrs.
3 rabbits	Dutch		1,750 mg. 7% aqueous soln., total dose 17,500–35,500 mg.[5380]	I.V	0	All survivors	Up to 4 yrs.
40 mice	C57BL		200 mg. powder once.[5384]	S.C	Tumors[5376, 5377]	All survivors sacrificed.	2 yrs.

[5373] Dextran 2; avg. molecular wt.: 100,000.
[5376] 810 controls with 6 reticulum cell sarcomas. Controls received various treatment. Survivor rates not reported.
[5377] 1 nodal reticulum cell sarcoma.
[5378] Dextran 5; avg. molecular wt.: "several million."
[5379] 1 lymphoma, 1 pulmonary adenoma.
[5380] 1 hepatic cholangioma, 2 hepatic reticulum cell sarcomas, 1 uterine carcinoma.
[5381] 200 controls with 11 reticulum cell sarcomas, 1 pulmonary adenoma, 3 mammary adenofibromas, 1 renal carcinoma, 4 uterine carcinomas, 1 ovarian carcinoma. Controls received various treatments. Survival rates not reported.
[5382] 1 hepatic reticulum cell sarcoma, 1 cardiac mesothelioma, 1 mammary adenofibroma.
[5383] 4 hepatic reticulum cell sarcomas, 2 pulmonary adenomas.
[5384] Dextran 5. Highly bronchial.
[5385] 3 nodal reticulum cell sarcomas.
[5386] Dextran 6. Highly bronchial.
[5387] Dextran 7. Highly bronchial.
[5388] Dextran 8; avg. molecular wt.: 37,500.
[5389] 1 lymphoma.
[5390] 1 hepatic cholangioma, 1 hepatic leukemia, 1 mammary adenofibroma, 1 uterine carcinoma.
[5391] 1 lymphoma, 1 leukemia, 2 hepatic reticulum cell sarcomas.
[5392] 2 nodal reticulum cell sarcomas, 1 hepatic reticulum cell sarcoma, 2 mammary adenofibromas, 2 uterine carcinomas.
[5393] Dextran 8; avg. molecular wt.: 37,000.
[5394] Dextran 9; avg. molecular wt.: 360,000.

Table 1 cont.

	Animal	Strain or type	Sex	Preparation and dose	Site and route	Animals with tumors	Survival	Duration of experiment
	20 rats	Bethesda Black		500 mg. powder once.[5256]	S.C	Tumors[5251, 5256]	All survivors sacrificed.	2 yrs.
	20 rats	Bethesda Black		300 mg. powder once.[5256]	I.P	Tumors[5251, 5256]	All survivors sacrificed.	2 yrs.
	3 rabbits	Dutch		1750 mg. 7% aqueous soln. Total dose 17,500–35,500 mg.[5258]	I.V	0	All survivors sacrificed.	Up to 4 yrs.
	20 mice	C57BL		200 mg. powder once.[5275]	I.P	Tumors[5275, 5287]	All survivors sacrificed.	2 yrs.
	40 mice	C57BL		200 mg. powder once.[5259]	S.C	Tumors[5275, 5259]	All survivors sacrificed.	2 yrs.
	20 mice	C57BL		200 mg. powder once.[5259]	I.P	Tumors[5259, 5275]	All survivors sacrificed.	2 yrs.
	20 rats	Bethesda Black		500 mg. powder once.[5258]	S.C	Tumors[5251, 5258]	All survivors sacrificed.	2 yrs.
Huener, W. C., 1959	20 rats	Bethesda Black.		500 mg. powder once.[5258]	I.P	Tumors[5251, 5258]	All survivors sacrificed.	2 yrs.
	3 rabbits	Dutch		1,750 mg. 7% aqueous soln. total dose 17,500–35,500 mg.[5258]	I.V	Tumors[5258]	All survivors sacrificed.	Up to 4 yrs.
	40 mice	C57BL		200 mg. powder once.[5258]	S.C	Tumors[5258, 5275]	All survivors sacrificed.	2 yrs.
	20 mice	C57BL		200 mg. powder once.[5258]	I.P	0[5275]	All survivors sacrificed.	2 yrs.
	20 rats	Bethesda Black.		500 mg. powder once.[5258]	S.C	Tumors[5251, 5258]	All survivors sacrificed.	2 yrs.
	20 rats	Bethesda Black.		500 mg. powder once.[5258]	I.P	Tumors[5251, 5267]	All survivors sacrificed.	2 yrs.
	3 rabbits	Dutch		1,750 mg., 7% aqueous soln. Total dose 17,500–35,500 mg.[5258]	I.V	Tumors[5258]	All survivors sacrificed.	Up to 4 yrs.

[5256] 340 controls with 6 reticulum cell sarcomas. Controls received various treatment. Survivor rates not reported.
[5251] 200 controls with 11 reticulum cell sarcomas, 1 pulmonary adenoma, 2 mammary adenofibromas, 1 renal carcinoma, 4 uterine carcinomas, 1 ovarian carcinoma. Controls received various treatments. Survival rates not reported.
[5258] Dextran 9; avg. molecular wt.: 300,000.
[5275] 2 uterine carcinomas, 1 ovarian cystadenoma.
[5259] 2 reticulum cell sarcoma, 2 hepatic reticulum cell sarcomas, 1 uterine carcinoma.
[5287] 1 lymphoma, 1 leukemia.
[5267] Dextran 10; avg. molecular wt.: 80,400.
[5260] 1 lymphoma, 2 hepatic reticulum cell sarcomas.
[5260] 4 hepatic reticulum cell sarcomas.
[5251] 1 nodal reticulum cell sarcoma. 1 hepatic cholangioma, 3 hepatic reticulum cell sarcomas, 1 mammary adenofibroma, 1 renal carcinoma, 1 uterine carcinoma.
[5287] 1 hepatic cholangioma, 2 hepatic reticulum cell sarcomas, 1 uterine carcinoma.
[5287] 1 lymphoma, 1 leukemia.
[5264] Dextran 11; avg. molecular wt.: 71,400.
[5264] 2 hepatic reticulum cell sarcomas, 1 leukemia.
[5258] 1 hepatic reticulum cell sarcoma, 1 uterine sarcoma.
[5267] 2 nodal reticulum cell sarcomas, 1 mammary adenofibroma, 1 uterine carcinoma.
[5260] 2 pulmonary adenomas.

The spleens of some mice and of one rabbit revealed marked mononuclear or reticulum-cell proliferations obliterating the lymph follicular structure of this organ. Two mice showed, moreover, pulmonary adenomas, one of which projected above the pleural surface as a polypous formation. The lung of one rabbit exhibited a large area of alveolar adenomatosis (461).

The various types of cancers found in dextran-treated animals were endothelioma of the endocardium, myeloid leukemia, reticulum-cell sarcoma of the liver or of the lymph nodes, and carcinoma or carcinosarcoma of the uterus. The author stated that it was noteworthy that this identity in histogenesis and histological structure of the cancers seen in dextran animals was shared only in part with those seen in normal control animals. The controls, while carrying some of the cancers recorded in the experimental animals, showed them at a considerably lower incidence rate and lacked also the various and frequent developmental and "precancerous" stages seen in the test animals (461).

Hueper concluded that some, but not all, dextrans elicit upon parenteral introduction into rats and mice, and perhaps also in rabbits, sarcomas originating from organs and tissues in which the substance is retained and stored, i.e., the reticuloendothelial tissues. Cancers from other tissues, such as uterus and skin observed in rats may be directly or indirectly caused by dextran. The experiments failed to provide information on physical or chemical molecular factors which could account for differences in carcinogenic potency of the various polymers (461).

In a study by Lusky and Nelson, 10 male and 10 female Osborne-Mendel rats and 10 male Bethesda Black rats were given weekly subcutaneous injections of 1 ml of 6% dextran (molecular weight not stated) for 73 weeks without the formation of any injection-site tumors. No influence on spontaneous-type tumor production was seen (647).

Two experiments by Richmond on the carcinogenicity of an iron-dextran complex included, as controls, tests of dextran. In the first experiment adult male rats were treated as follows (830):

(a) 40 received a weekly intramuscular injection of 0.4 ml iron-dextran complex into the right upper thigh. Each dose contained 20 mg of iron as ferric hydroxide in complex with low-molecular-weight dextran.

(b) 12 received weekly intramuscular injections of 0.5 ml "ferrivenin" (Benger) under ether anaesthesia, with occasional interrupations owing to the development of ulceration. Each dose contained 10 mg of iron as saccharated oxide of iron.

(c) 12 received weekly injections of 0.5 ml low-molecular-weight dextran.

(d) 12 received weekly injections of 0.5 ml normal saline solution.

In the second experiment weanling rats weighing an average of 48 g were treated as follows (830):

(a) 20 males and 20 females were given twice-weekly intramuscular injections of iron-dextran complex into the right upper thigh in graduated dosage according to weight--namely, 0.1 ml up to 100 g, 0.2 ml up to 150 g, 0.3 ml up to 200 g, and 0.4 ml thereafter. This regimen was stopped after three months, each animal having received 9.5 ml iron-dextran complex.

(b) 6 males and 6 females received corresponding volumes of low-molecular-weight dextran solution.

In both experiments a highly significant number of the rats receiving iron-dextran developed injection site tumors, while neither group of rats receiving dextran alone nor any of the controls developed any tumors at the site of injection (830).

Numerous studies, many of which appear to contradict each other, have been conducted on the effects of intravenously administered dextran on tumor promotion in animals inoculated with tumor cells. These studies provide little information relative to the evaluation of a food additive and, therefore, only a few representative studies have been included in this monograph.

The incidence and size of hepatic metastases following intraportal injection of known numbers of Walker carcinoma cells to female Sprague-Dawley rats were found by Fisher and Fisher to be increased after i.v. administration of low, medium, and high molecular weight dextrans. The dextrans were not hepatotoxic, and their effect on tumor growth appeared to be unrelated to alterations they induced in viscosity of the blood or hematocrit. A simple suspension of tumor cell inocular in low molecular weight dextran also resulted in augmentation of tumor growth. The investigators observed in their studies a consistent and roughly quantitative relationship between tumor growth and increase in circulating blood volume resulting from the administration of low molecular weight dextran or other modalities such as plasma or saline infusions (281).

In another study by Fisher and Fisher, the i.v. administration of low molecular weight dextran to rabbits receiving intraportal inoculations of V2 carcinoma cells resulted in a slight increase in the incidence of lung metastases and a significant enhancement of the incidence and growth of hepatic metastases (282).

Hagmar tested the effect of low molecular weight dextran on intravenously-induced metastases in a syngeneic tumor-host system in mice. When given as intravenous pretreatment the dextran increased the total number of gross extrapulmonary metastases, without significantly changing the planimetrically estimated lung and liver metastases. When, on the other hand, the same volume of dextran was given in the cell suspension, there was a further increase in gross extrapulmonary metastases and also in pulmonary metastases (393).

Wood et al. reported that the intravenous administration of three fractions of low molecular weight dextran to Laboratory Lop rabbits before or after i.v. inoculation of ascitic V2 carcinoma cells (see Table 2) failed to significantly alter the frequency of pulmonary metastases. No extrapulmonary tumors were found in any of the animals (1094).

TABLE 2. Effect of Dextran on Lung Metastasis

Group	No. of rabbits	Dose ml/kg	Mean no. lung tumors ± SE*
Experiment I			
Control (5% dextrose in water)	13	15.0	91.62 ± 9.10
Dextran-10 (pretreatment)	14	15.0	83.86 ± 7.91
Dextran-10 (post-treatment)	12	15.0	91.33 ± 10.77
Experiment II†			
Control (5% dextrose in water)	13	26.0	58.80 ± 3.86
Dextran-10 (post-treatment)	15	72.5	11.71 ± 4.29
Experiment III			
Control (5% dextrose in water)	13	19.5	11.29 ± 1.55
Dextran-70 (post-treatment)	14	32.5	12.60 ± 1.69
Dextran-10 (post-treatment)	14	32.5	11.93 ± 1.99
Experiment IV			
Control (saline)	14	15.2	67.71 ± 4.87
Dextran-70 (post-treatment)	14	15.2	55.86 ± 6.17

* Standard error.

† In this experiment the level of statistical significance for the control vs. treated group revealed $P < 0.05$.

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption - Distribution

No Information Available

III. Metabolism and Excretion

Scully et al. reported that preliminary tests by a number of investigators using radioactive dextran (unspecified route of administration) definitely indicate that dextran is metabolized by mice, rats, dogs and human beings, with a substantial portion of the labeled dextran appearing in the expired air as $^{14}CO_2$ (903).

Several workers have shown that extracts of various tissues of different mammals, including man, possess dextranase activity, the activity being localized primarily in the small-intestine mucosa (14, 100, 195, 196).

In a study by Bloom and Wilhelmi, the administration by stomach tube of 5 ml of 18% dextran in 0.9% saline to 250-350 g male Sprague-Dawley rats, which had been fasted for 24 hours, led to a significant increase in the liver glycogen 4 hours after feeding (see Table I). To show that glycogen was indeed present in the liver rather than dextran, some of the samples were allowed to stand at room temperature for 1.5-2 hours in order that glycogenolysis might occur. This resulted in almost complete disappearance of the glycogen, while dextran added to parallel samples was recovered almost quantitatively (see Table II). Blood samples taken from the rats before and after administration of dextran showed a substantial increase in blood sugar following the feeding. In a similar study, 2 human subjects exhibited an increase in blood sugar following oral administration of 100 ml of 20% dextran (100).

TABLE I. Liver Glycogen Concentration in Fasting and Dextran Fed Rats.

	Alkali soluble, mg/100 g	TAA soluble, mg/100 g
10 fasting rats	130 ± 13*	31 ± 8
14 fasted rats 4 hr after dextran feeding		
Immediate analysis	751 ± 106	469 ± 66
Aliquot at room temp. 2 hr	71 ± 13	10 ± 2

* Stand. error.

TABLE II. Recovery of Added Dextran from Livers Undergoing Glycogenolysis.

No. of livers analyzed	Dextran added, mg/g	Dextran recovered, mg/g	
		Alkali soluble	TAA soluble
6	644	517 ± 31*	610 ± 8
8	736	720 ± 22	688 ± 20

* Stand. error.

The dextran was added to each of two samples, one of which was digested with alkali, the other of which was extracted with trichloroacetic acid. Time of incubation at room temp. was 2 hr.

Following oral administration of 500 mg of dextran to fasting male Sprague-Dawley rats, Parkinson observed about a 6-fold increase in the liver glycogen (772).

Baker stated, without supporting data, that while dextran is edible and assimilated without unfavorable effect on the human system, it appears that the alpha-(1-6) linkages are resistent to attack by bacteria and enzymes present in the gastro-intestinal tract. He also stated that biological tests (unspecified test animals) have demonstrated that when dextran containing a high proportion of alpha-(1-6) linkages is included in a normal diet on a regular regimen, gain in body weight is inhibited (54).

IV. Effects on Enzymes and Other Biochemical Parameters

No Information Available

V. Drug Interaction

No Information Available

VI. Consumer Exposure

Dextran is used as an additive to sugar-containing products, such as sirups and candies, to increase the moisture retentivity, improve body, and inhibit crystallization of the sugar. A film of dextran is used to coat foods such as meats, dried fruits and cheese to protect the food against drying in storage.

DEXTRANS

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Fed. Proc. 16: 143. 1957.

518. A DEXTRAN-HYDROLYZING ENZYME FROM INTESTINAL MUCOSA. George A. Adrouny,* Walter L. Bloom* and Alfred E. Wilhelmi. Dept. of Biochemistry, Div. of Basic Health Sciences, Emory Univ., Ga.

Extracts prepared from the mucosa of the small intestine of various mammalia - rat, guinea-pig, rabbit, pig, cow, dog and man - were found to induce an increase in the reducing power of dextran, a 1,6-glucosidic polysaccharide. Comparison of crude extracts from the various sources showed the preparation from the rat to be 60-80 times more active than those from the pig and the cow, while the activities of material from other species appeared to be at intermediate levels. Further experiments were therefore mostly carried out with extracts from the rat. Maximal activity was seen at pH 6.0-6.2. Preparations retained most of their activity for about 3 wk, if preserved below 4°C, at a pH of 6.0-7.0. Deterioration was very fast at a low pH, somewhat slower in alkaline media. The active principle is highly soluble in water; 50% saturated ammonium sulfate or 30-40% acetone, methanol or ethanol retain about 50% of the initial activity in solution. Other substrates that are hydrolyzed by crude or partially purified preparations include isomaltose, beta amylase limit dextrin, glycogen and starch; yeast mannan is not affected. Invertase accompanies "dextranase" in all fractions. Fractionation by organic solvents, adsorption on calcium phosphate, norit and diethylaminoethyl cellulose, precipitation with Zn^{++} and UO_2^{++}, and zone electrophoresis at various pH's were ineffective for the separation of the two principles. However almost complete disappearance of invertase activity, with retention of about 90% "dextranase" activity, could be achieved by precipitation of the invertase with Hg^{++}.

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Dextran as a Source of Liver Glycogen and Blood Reducing Substance.*. (19922)

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In earlier experiments in this laboratory it has been observed that dextran, fed to human subjects, is not recovered in the stools. Engstrom and Aberg(1) have suggested that some of the dextran administered intravenously might be excreted into the gastrointestinal tract where it could be destroyed by bacterial action. Our own observations on dextran incubated with stools suggested that dextran disappears only very slowly, but Hehre(2) has since shown that there are large numbers of anaerobic bacteria present in the intestinal flora, and that it is these, rather than the aerobes, that are capable of splitting dextran. Although ingested dextran may be in part degraded and consumed by the intestinal bacteria, it still seemed to us of interest to learn more about the disappearance of dextran fed by mouth. The following experiments show that dextran feeding leads to a sustained increase in blood sugar and a rise in liver glycogen in the fasted rat, and brings about an

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TABLE I. Blood Reducing Substance Following Administration of 5 ml of 18% Dextran by Stomach Tube to 24-Hr Fasted Rats.

Time in hr	0	1	2	3	4
No. of rats	6	4	4	2	2
Reducing substance (mg %)	80 ± 7.9	113 ± 3.5	100 ± 8.2	96.5 ± 2	95.5 ± 2

increase in the blood sugar of human subjects.

Experimental. Male albino rats of the Sprague-Dawley strain, weighing 250-350 g were fasted for 24 hours. A group of 10 control animals were killed and both fractions of liver glycogen were determined by the method of Bloom, Lewis and Schumpert(3). Glycogen was determined (as glucose equivalent) by means of the anthrone reaction. The fasted experimental animals were given 5 ml of 18% dextran in 0.9% saline by stomach tube. An initial blood sample was taken from the cut end of the tail and additional samples were taken at intervals after feeding. Copper tungstate filtrates were prepared and reducing substances were determined by the method of Nelson(4). In a series of four rats, both total and fermentable reducing substances were determined. Two or four hours after feeding, the rats were anesthetized with Nembutal and the liver was removed and immediately frozen and powdered. Trichloroacetic acid-extractable and total glycogen was determined on aliquots of the well-mixed liver powder. In order to determine whether glycogen or dextran might be present, other samples were allowed to stand at room temperature for 1.5 to 2 hours in order that glycogenolysis might occur. Dextran was added to parallel samples in order to determine whether dextranolysis could occur in the liver preparation.

To two human subjects, fasted for 12 hours, 100 ml of 20% dextran were given orally and blood sugar was determined at 0, 0.5, 1 and 2 hours thereafter. Two other patients were fasted 12 hours, 200 ml of 20% was given orally and blood sugars determined at the described intervals.

Results. The data of Table I show that the blood reducing substance in dextran-fed rats increases significantly in one hour after feeding and is still above the initial level after four hours. In Table II it is seen that the increase in blood reducing substance (of about the same extent but of shorter duration after feeding half the amount of dextran given to the first series of animals) is entirely fermentable. Table III presents the data on two human subjects given 20 g of dextran by mouth. The increases in blood sugar in 0.5 to 1 hour are of the same order as those seen in the rats.

In Table IV it is seen that there is a considerable increase in liver glycogen four hours after feeding dextran to previously fasted rats, and that allowing the liver samples to stand

TABLE II. Total and Non-Fermentable Blood Reducing Substance in 24-Hr Fasted Rats Given 5 ml of 9% Dextran by Stomach Tube.

Wt, g	Time after dextran	Blood reducing substance (mg %) Total	Non-fermentable
285	Control	99.2	0
	30 min.	116.2	0
	2 hr	86.1	0
	4 "	73.2	0
300	Control	80.1	0
	30 min.	121.7	0
	2 hr	74.8	0
	4 "	63.6	.25
265	Control	87.4	0
	30 min.	104.6	0
	2 hr	81.8	2.5
	4 "	67.7	.5
250	Control	65.5	12.7
	30 min.	97.6	0
	2 hr	81.8	4.3
	4 "	68.5	1.3

TABLE III. Blood Reducing Substance Following Administration of 100 ml of 20% Dextran to 12-Hr Fasted Human Subjects. (Values for reducing substance in mg %.)

	Subject I	II	III	VI
Control	83	100	90.5	118
½ hr	107	110	103	161
1 "	98	126	101	137
2 "	89	112	104	128

TABLE IV. Liver Glycogen Concentration in Fasting and Dextran Fed Rats.

	Alkali soluble, mg/100 g	TAA soluble, mg/100 g
10 fasting rats	130 ± 13*	31 ± 8
14 fasted rats 4 hr after dextran feeding		
Immediate analysis	751 ± 106	469 ± 66
Aliquot at room temp. 2 hr	71 ± 13	10 ± 2

* Stand. error.

TABLE V. Recovery of Added Dextran from Livers Undergoing Glycogenolysis.

No. of livers analyzed	Dextran added, mg/g	Dextran recovered, mg/g	
		Alkali soluble	TAA soluble
6	644	517 ± 31*	610 ± 8
8	736	720 ± 22	683 ± 20

* Stand. error.

The dextran was added to each of two samples, one of which was digested with alkali, the other of which was extracted with trichloroacetic acid. Time of incubation at room temp. was 2 hr.

for 2 hours at room temperature results in the disappearance of most of the glycogen. Dextran added to aliquots of the liver tissue and determined either as alkali-soluble or trichloroacetic acid-soluble anthrone-reacting substance, is recovered without substantial loss, so that no significant dextranolysis seems to occur under these conditions in liver tissue (Table V).

Discussion. The data show that in both rat and man the oral administration of dextran leads to a significant and sustained increase in blood reducing substance, most of which is fermentable. In the rat, this increase in blood sugar is accompanied by a significant increase in liver glycogen 4 hours after feeding. Dextran is therefore capable of being broken down in the intestine to products which yield glucose and glycogen in the animal. The early increase in blood sugar in both rat and man indicates that the intestinal breakdown of dextran may be a relatively rapid process, and it suggests that this may not be ascribable merely to bacterial action but more probably to an action of an enzyme or enzymes of the intestinal tract. Experiments now under way in this laboratory indicate that the latter possibility may be realized: suspensions of rat duodenal mucosa have been found to liberate glucose from dextran at rates which can account satisfactorily for the increases in blood sugar seen after feeding.

The authors wish to express their appreciation to Dr. Conrad Kinard who helped with the clinical study.

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The Location of Carbohydrases in the Digestive Tract of the Pig

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Little is known about the locations in the mammalian intestine at which hydrolysis of disaccharides and polysaccharides occurs.

In a recent investigation of intestinal digestion and absorption in man, Borgström, Dahlqvist, Lundh & Sjövall (1957) observed that the invertase activity (in unpublished experiments we have demonstrated that the maltase and lactase activities are distributed similarly) of the intestinal contents, which was weak throughout the whole small intestine, was maximal in the lower part of the small intestine (lower jejunum and ileum). In spite of this, the absorption of lactose occurred in the upper part of the small intestine (duodenum and upper jejunum), where no disaccharidase activity could be demonstrated in the intestinal contents (Ammon & Henning, 1956; Borgström *et al.* 1957). The hydrolysis of disaccharides during their absorption is catalysed by enzymes apparently situated inside the cells of the intestinal mucosa (Borgström *et al.* 1957). Little information is, however, available about the relative disaccharidase activity of the mucosa of different parts of the small intestine.

Amylase, in contrast to the disaccharidases, is mainly secreted into the intestinal lumen in the

pancreatic juice. However, preparations from the intestinal mucosa also contain amylase. Intestinal-mucosa preparations from different species of mammals have been reported to contain one further polysaccharidase, namely dextranase (Adrouny, Bloom & Wilhelmi, 1957). It does not seem to be known, however, whether dextranase is present in the pancreas too.

This paper records the carbohydrase activities of homogenates of mucosa from different parts of the small intestine, from the stomach and from the colon, and of a homogenate of pancreatic tissue. The adult pig was selected as an experimental animal.

EXPERIMENTAL

Determination of enzymic activities

Disaccharidase activities. These were measured by the methods described previously (Dahlqvist, 1960*d*). One unit of disaccharidase activity causes 5% of hydrolysis of the particular disaccharide in 2·0 ml. of reaction mixture at 28 mM-substrate concentration in 60 min. at 37°.

Amylase activity. The substrate solution was prepared by dissolving 2·0 g. of soluble starch a.m. Zulkowsky (from Merck A.G., Germany) and 40 mg. of NaCl in 0·05M-phosphate buffer, pH 6·9 (3·026 g. of KH_2PO_4 and 3·959 g. of $Na_2HPO_4,2H_2O$/l.), to a final volume of 100 ml. Toluene (1 ml.) was added as a preservative and the solution was stored in a refrigerator. The substrate solution was prepared weekly.

For determination of amylase activity, 1·0 ml. of suitably diluted enzyme solution was mixed with 1·0 ml. of substrate solution and immersed in a water bath at 37°. After 60 min. the reaction was interrupted by the addition of 2·0 ml. of dinitrosalicylate reagent (prepared as described by Hostettler, Borel & Deuel, 1951). A blank was prepared with the same composition, in which, however, the 3:5-dinitrosalicylate reagent was added immediately after the mixing of the enzyme and substrate. The tubes were immersed in a boiling-water bath for 10 min. and then chilled for 2 min. with running tap water. After dilution with 20·0 ml. of water the intensity of the red colour produced was measured in a Beckman B spectrophotometer at a wavelength of 530 mμ, in 1 cm. cuvettes. A standard curve was prepared from known solutions of maltose (the tubes containing 0·5–2·0 mg. of maltose monohydrate). One unit of amylase activity causes an increase of reducing power corresponding to 1 mg. of maltose monohydrate in 60 min. during these conditions. If the increase of reducing power does not exceed that of 2 mg. of maltose monohydrate, the enzymic reaction follows zero-order kinetics and the amount of reducing groups liberated is proportional to the amount of enzyme present.

The amylase unit, when defined in this way, is comparable with the unit used for disaccharidase activity (Dahlqvist, 1960*d*). It should not, however, be confused with the amylase unit used by Borgström *et al.* (1957), since in that case the incubation was performed for 3 min. at 25°.

Dextranase activity. The substrate solution for determinations of dextranase activity was prepared by dissolving 2·0 g. of dextran (dextran 40, mol.wt. by light-scattering 41 000, by end-group analysis 26 000, obtained from Pharmacia A.B., Sweden) in 0·1M-maleate buffer (Gomori, 1955), pH 6·0, to a final volume of 100 ml. Incubation and determination of the degree of hydrolysis were performed in exactly the same way as for determinations of amylase activity. Maltose was used for preparation of the standard curve, since maltose and isomaltose have the same extinction coefficient with the 3:5-dinitrosalicylate reagent. One unit of dextranase activity is the amount of enzyme which causes an increase of reducing power equal to that of 1 mg. of maltose monohydrate in 60 min.

Determination of protein

The method of Lowry, Rosebrough, Farr & Randall (1951) was employed, the modified reagent B introduced by Eggstein & Kreutz (1955) being used. A standard curve was prepared with human serum albumin (kindly supplied by A. B. Kabi, Sweden).

Preparations of homogenates

The stomach, small intestine, upper part of the colon and pancreas of an adult pig were cut out immediately after slaughter and chilled with crushed ice during transport to the laboratory. From pieces of stomach, small intestine and colon the mucosa was scraped off with a glass slide and homogenized in an Ultra-Turrax homogenizer for 2 min. with an equal weight of 0·9% NaCl. A piece of the pancreas was homogenized in the same way. This method has earlier been found suitable for the extraction of glycosidases from hog small-intestinal mucosa (Borgström & Dahlqvist, 1958). After centrifuging in a Wifug laboratory centrifuge for 5 min., the opalescent supernatant was assayed for carbohydrase activities.

RESULTS

There are several ways of expressing the relative carbohydrase activities of the different segments of the intestine. Some authors have expressed the activity per cm.² of intestine (Euler & Svanberg, 1921). Because of the enormous surface area of intestinal villi, as well as their variation in density along the length of the intestine, it seems more logical to compare the activity per gram of mucosa (Cajori, 1935; Heilskov, 1951). Since in the present investigation the homogenates have always been prepared from a mixture of equal weights of mucosa and 0·9% NaCl, the carbohydrase activities can be compared directly when expressed per ml. of homogenate preparations.

The protein content of the homogenates varied (Table 1), however, and therefore in Figs. 1–5 the specific carbohydrase activity (i.e. number of units/mg. of protein) has been used to enable comparison of the relative carbohydrase activities of the different segments of the small intestine. However, the results are similar whether enzyme activity is presented as units/mg. of mucosal protein or units/ml. of mucosal homogenate. The experimental conditions for carbohydrase assay were such that 0·5 unit/ml. of each of the activities investigated could be detected.

To check the completeness of the removal of the mucosa with the glass slide, in one experiment the remaining intestinal wall was also homogenized, and the homogenate was assayed for invertase activity. It was found that 80% of the total amount of invertase originally present in the piece of intestine had been removed with the mucosa.

Stomach. No invertase, maltase, isomaltase, trehalase, lactase, cellobiase or dextranase activity could be detected. The preparation had very weak amylase activity (17 units/ml.; cf. below), which may very well have been caused by contamination with saliva from the gastric contents. The mucosa of the stomach therefore does not seem to contain any carbohydrases.

Small intestine. This had a total length of 16 m. The duodenum had a length of 0·34 m., the jejunum and the ileum had each a length of about 8 m. In the duodenum and jejunum no intestinal contents had to be removed, but in the ileum the contents were removed and the intestine was gently blotted with a piece of cloth before the mucosa was scraped off. The pieces of the small intestine selected are noted in Figs. 1–5. The amount of mucosa obtained from 50 cm. of small intestine varied between 7 and 11 g.

Invertase activity was low in the duodenum (7·5 units/ml. of homogenate) and was greatest in the lower jejunum and the ileum (50–70 units/ml.) (Fig. 1). Even the homogenate prepared from a section of the lower ileum, cut just proximal to the ileocoecal valve, had strong invertase activity (54 units/ml.).

Maltase activity of the homogenate from the duodenum was 66 units/ml., which is nearly 10 times its invertase activity. The maltase activity also increased in the lower part of the small intestine (Fig. 1) but not to the same extent as the invertase activity. In the homogenates from the lower jejunum and the ileum the maltase activity was 100–150 units/ml., which is two to three times the invertase activity.

The maltase activity of the small intestine of the pig has recently been demonstrated to be effected by a mixture of three different maltases (maltase I–III). The results of the determination of each of these enzymes, by the methods described earlier (Dahlqvist, 1959, 1960*c*, *d*), with 28 mM-substrate

Table 1. *Protein content of the homogenates*

All tissues were homogenized with one part (w/w) of 0·9% NaCl soln.

Source of homogenate	Protein (mg./ml.)
Stomach	11·5
Duodenum	56·0
Upper jejunum	63·0
Lower jejunum	41·0
Upper ileum	30·0
Lower ileum	34·0
Colon	25·0
Pancreas	45·0

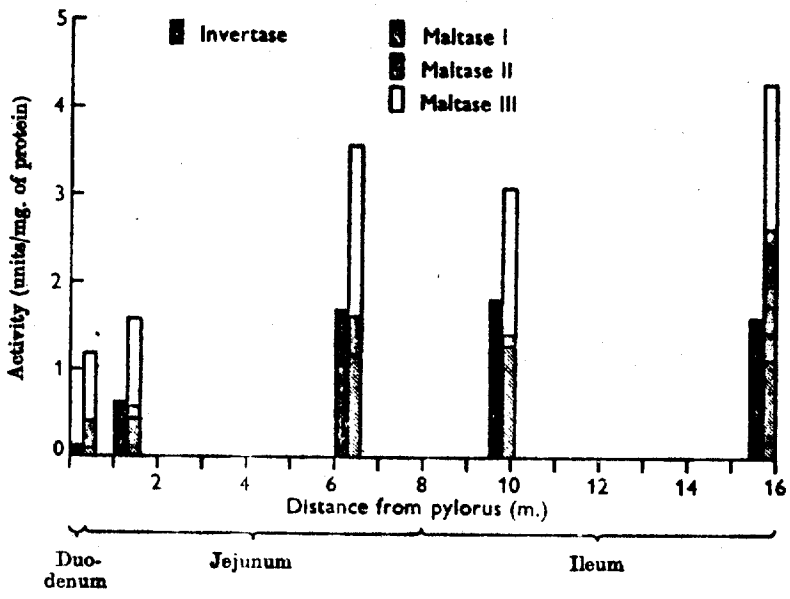

Fig. 1. Invertase and maltase activities of homogenates of mucosa from different parts of the small intestine of an adult pig. The three different maltases (maltase I–III) present in such preparations have been measured separately by the methods described earlier (Dahlqvist, 1959, 1960*c*, *d*).

concentration (Dahlqvist, 1960*d*), are seen in Fig. 1. Maltase II activity, as compared with maltase III, is unusually low. Other experiments with homogenates prepared from the small intestine of the pig have demonstrated that maltase II activity is usually equal to or somewhat greater than maltase III activity; maltase II and maltase III together usually exert 80–85 % of the total activity (Dahlqvist, 1960*d*).

Isomaltase activity, which is to some extent due to the presence of maltase II and maltase III but mostly (75 %) to a 'specific' isomaltase (Dahlqvist, 1960*b*), had a distribution in the small intestine which was similar to that of the invertase and maltase activities (Fig. 2), i.e. the isomaltase activity per ml. of homogenate rose from a low value (1·5 units/ml.) in the preparation from the duodenum to about 30 units/ml. in the homogenates from the lower jejunum and the ileum.

Trehalase activity, which is due to a specific enzyme (Dahlqvist, 1960*a*), showed a distribution which was quite opposite to that of the enzymes described above (Fig. 3). The trehalase activity was greatest in the preparations from the duodenum and the jejunum (15–20 units/ml. of homogenate) but decreased in the lower part of the small intestine.

Lactase activity was greater in the homogenate from the upper jejunum (18 units/ml.) than in that from the duodenum (9 units/ml.), but was practically absent from the homogenates of the ileum (Fig. 4). The cellobiase activity had the same distribution as the lactase activity, and the ratio of cellobiase to lactase was the same in homogenates from all parts of the small intestine (Fig. 4). This suggests the possibility that the cellobiase and lactase activities are caused by one and the same enzyme. The relation between these two activities is under investigation in our laboratory.

Amylase activity was present in all the homogenates of small-intestinal mucosa, but was low (200–700 units/ml.) when compared with the activity in the homogenate of the pancreas (see below). It is quite possible that at least some of the activity of the mucosa homogenate was caused by contamination with intestinal contents, which contain considerable amounts of pancreatic amylase.

The amylase activity of the homogenates, prepared from different parts along the small intestine, varied irregularly.

Dextranase activity was low in all the homogenates, but increased in a distal direction in the small intestine from 1 unit/ml. in the homogenate from the duodenum to 5·5 units/ml. in that from the lower ileum (Fig. 5). Since the homogenate of the pancreas had no dextranase activity (see below) the activity of the homogenates of intestinal mucosa could not be caused by contamination with pancreatic juice. The low dextranase activity of the samples, together with the localization of this activity to the distal part of the small intestine, might suggest that the dextranase activity was

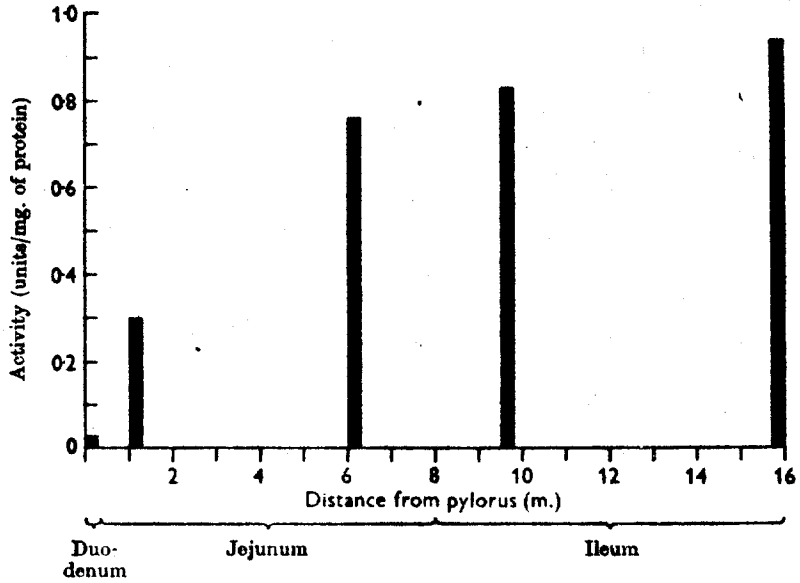

Fig. 2. Total isomaltase activity of homogenates of mucosa from different parts of the small intestine of an adult pig. The isomaltase activity of such preparations is exerted by a mixture of three different enzymes as described previously (Dahlqvist, 1960*b*).

caused by contamination with intestinal bacteria. Against this possibility is, however, the low dextranase activity of the homogenate of mucosa from the colon (Table 2).

Colon. The mucosa from the colon was scraped off a few centimetres distal to the ileocoecal valve. The carbohydrase activities of the homogenate are seen in Table 2. Although most of the carbohydrase activities were present in this preparation too, they were present only in small amounts, as compared with the activities of the homogenates of small intestinal mucosa.

Pancreas. The homogenate of the pancreas was kept chilled with crushed ice until just before assay, and all assays were completed within a few hours after the homogenization.

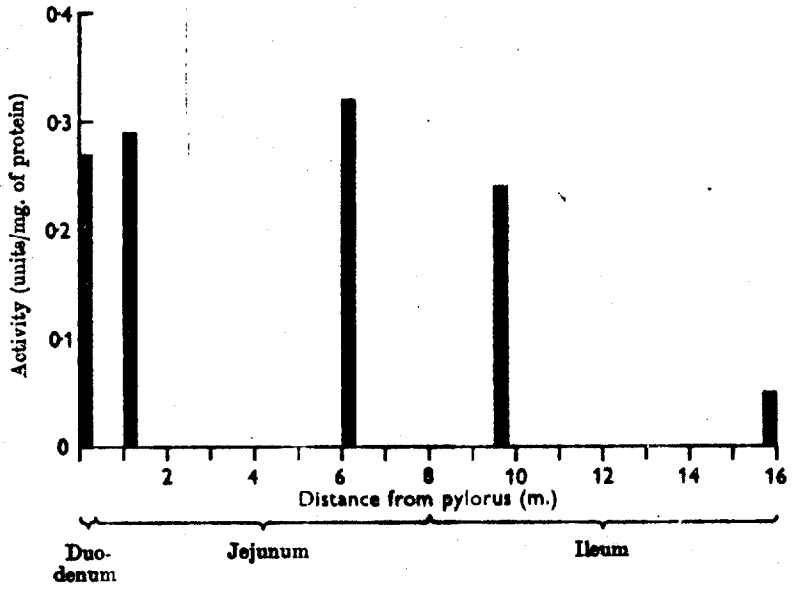

Fig. 3. Trehalase activity of homogenates of mucosa from different parts of the small intestine of an adult pig.

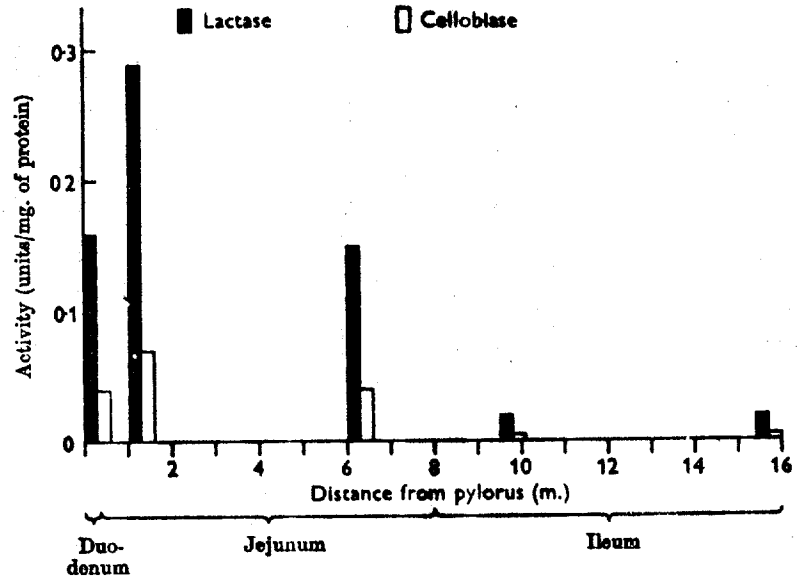

Fig. 4. Lactase and collobiase activities of homogenates of mucosa from different parts of the small intestine of an adult pig.

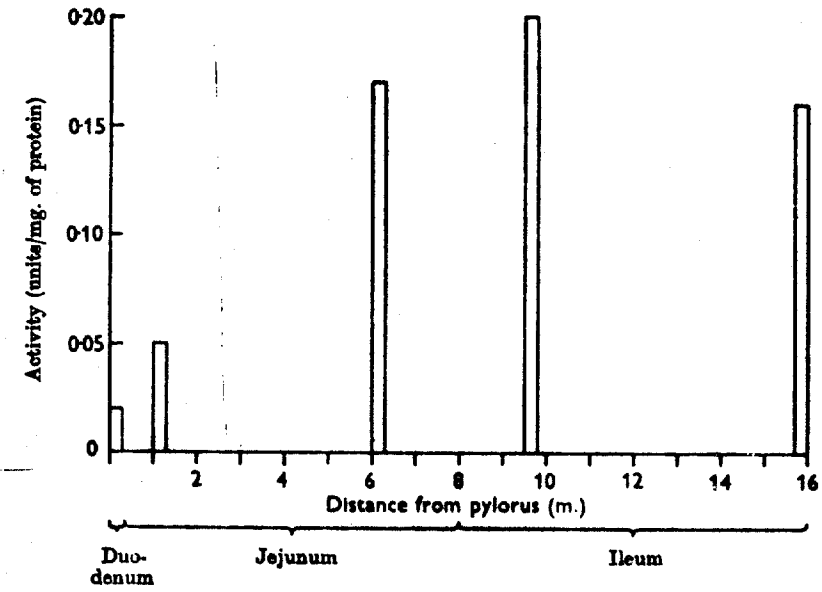

Fig. 5. Dextranase activity of homogenates of mucosa from different parts of the small intestine of an adult pig.

Table 2. *Carbohydrase activities of the homogenate of mucosa from the colon of an adult pig*

Protein content of the homogenate was 25 mg./ml.

Enzyme	Activity (units/ml. of homogenate)
Invertase	8
Maltase I	6
Maltase II	18
Maltase III	0
Isomaltase	2
Trehalase	0·5
Lactase	0
Cellobiase	0
Amylase	250
Dextranase	1

Only two carbohydrase activities were recognized, namely very active amylase (80 000 units/ml.) and weak maltase (12 units/ml.) activity. This amylase activity was more than 100 times that of the homogenate of small-intestinal mucosa, which suggests that it is mainly pancreatic amylase which effects the hydrolysis of starch in the small intestine.

The pancreatic-maltase activity, by contrast, was very weak, whether compared with that of the preparations of small-intestinal mucosa (see above) or with the pancreatic-amylase activity. It may therefore be concluded that the pancreatic maltase is without physiological importance for the hydrolysis of maltose in the small intestine.

DISCUSSION

It is apparent that the disaccharidases are mainly localized in the small intestinal mucosa. The weak maltase activity of the pancreas and the weak disaccharidase activities of the mucosa of the colon cannot contribute to any considerable extent to the hydrolysis of ingested disaccharides.

The findings about the location of the disaccharidase activities along the small intestine seem remarkable. From the present investigation it appears that there exist two major groups of pig-intestinal disaccharidase activities, according to their distribution along the small intestine. One group (invertase, maltase and isomaltase) is localized mainly in the distal part of the small intestine, and the other group (trehalase, lactase and cellobiase) is localized mainly in the proximal part of the small intestine. It seems tempting to conclude that the corresponding disaccharides are hydrolysed and absorbed in the corresponding parts of the small intestine. Our previous finding that lactose is absorbed in the proximal part of the small intestine of humans (Borgström *et al.* 1957) agrees with this idea, but further experimental studies are required.

The location of the invertase activity differs from the findings by some earlier authors who have studied other species. Euler & Svanberg (1921) analysed a human small intestine and found that the invertase activity (per cm.^{2} of intestine) was highest in the upper part of the jejunum, whereas

the lower ileum had only 5% of this activity. Recently Ammon & Henning (1956) have found a similar distribution of invertase in rabbits. In the present paper figures are reported for one pig only. Experiments with pieces of intestine of several other pigs have, however, demonstrated that there are hardly any individual variations in the location of the different carbohydrases.

The location of the lactase activity along the small intestine agrees well with that given in earlier reports. Cajori (1935) has found that the lactase activity of mucosa preparations from dog small intestine was 30% greater in preparations from the jejunum than in those from the duodenum (calculated per gram of mucosa), and Heilskov (1951) has found that the lactase activity of the mucosa in different mammals (rabbits, cows and human foetuses) diminishes in the distal segments of the small intestine.

That the homogenate of the pancreas contained a powerful amylase but no disaccharidases, except a very weak maltase, is in accordance with earlier reports, which state that the pancreas contains amylase and maltase, but no invertase (Brown & Heron, 1880; Oppenheimer, 1925), lactase (Heilskov, 1951) or trehalase (Frèrejacque, 1953). The absence of disaccharidase activities from the pancreatic juice can also be deduced from the finding that the small-intestinal contents contain amylase but essentially no disaccharidases (Ammon & Henning, 1956; Borgström *et al.* 1957).

The fact that the homogenate of the pancreas had no dextranase activity demonstrates that intestinal dextranase is a specific enzyme, distinguished from amylase. Like the disaccharidases the dextranase is located in the intestinal mucosa.

SUMMARY

1. The carbohydrase activities of homogenates prepared from mucosa of the stomach, small intestine and colon, and from pancreatic tissue, of an adult pig have been studied.

2. The preparation from the stomach did not contain any carbohydrases except a very weak amylase activity, which may have been caused by contamination with saliva from the gastric contents.

3. The preparations from the small intestine had powerful disaccharidase activities which showed different locations along the small intestine: one group of activities (invertase, maltase and isomaltase) was mainly localized in the distal part of the small intestine, and another group (trehalase, lactase and cellobiase) was localized in the proximal part. This may indicate that different disaccharides are absorbed in different parts of the small intestine.

4. The preparation from the small intestine also had amylase activity, which, however, was low compared with that of the pancreas homogenate and may have been due to contamination with pancreatic juice.

5. Dextranase activity was present in the preparations from the small intestine, but not in those from the pancreas. The dextranase activity is thus caused by a specific enzyme which has its origin in the small intestinal mucosa.

6. The preparation from the colon also had carbohydrase activities, although these activities were weak compared with those of the small intestinal mucosa.

7. The preparation from the pancreas had very powerful amylase activity, and the pancreas seems to be the main source of the amylase of the intestinal contents. This preparation also had weak maltase activity. The preparation from the pancreas had no other disaccharidase activities.

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Rat-Intestinal Dextranase

LOCALIZATION AND RELATION TO THE OTHER CARBOHYDRASES OF THE DIGESTIVE TRACT

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Extracts of the small-intestinal mucosa of different mammals, including man, possess dextranase activity (Adrouny, Bloom & Wilhelmi, 1957; Dahlqvist, 1961*a*, 1962). The dextranase activity seems to be sufficiently strong to enable a rapid utilization of ingested dextran (Bloom & Wilhelmi, 1952). The intestinal dextranase may also be of importance for the metabolism of dextran which has been administered parenterally as a plasma substitute, since this is known to be secreted into the gastrointestinal tract (Åberg, Bloom & Hansson, 1961). Dextran can, however, be hydrolysed in other parts of the body also, since several different tissues contain dextranase (Rosenfeld & Lukomskaya, 1956, 1957; Lukomskaya & Rosenfeld, 1958; Fischer & Stein, 1960).

The subject of the study reported here is the distribution and the specificity of the dextranase present in the digestive tract. Since the intestine of the rat has especially high dextranase activity (Adrouny *et al.* 1957) this animal was selected for the study.

MATERIALS AND METHODS

Determination of carbohydrase activities

Dextranase and amylase activities. The dextranase and amylase activities were measured by the methods used for the pig enzymes by Dahlqvist (1961*a*).

In the present paper the unit of amylase and dextranase activities has been defined as the activity liberating reducing groups corresponding to 1 μmole of maltose (isomaltose)/min. at 37°, instead of 1 mg./hr. as in the previous investigation. This is in accordance with recommendations recently made by the joint Sub-Commission on Clinical Enzyme Units of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry (Freeman, 1961). One unit defined in this way equals 21·6 units of those previously used.

Disaccharidase activities. For the assay of disaccharidase activities the tris-buffered glucose-oxidase reagent was used (Dahlqvist, 1961*b*). The incubation conditions were those used for the pig enzymes (Dahlqvist, 1960*b*, 1961*b*) but the unit is now defined as the activity hydrolysing 1 μmole of disaccharide/min. at 37° instead of 1 mg./hr. One unit thus defined equals 21·6 of the units previously used.

Determination of protein

Protein determinations were performed with the method of Lowry, Rosebrough, Farr & Randall (1951), with the modified 'reagent B' introduced by Eggstein & Kreutz (1955). A standard curve was prepared with freshly dissolved human serum albumin, kindly supplied by A. B. Kabi (Stockholm, Sweden).

Animal preparations

Rats of either sex, weighing 200–250 g., of the Sprague-Dawley strain were used.

Tissue homogenates. The rats were starved for 18–24 hr., with free access to water. Each rat was killed, the abdomen opened and the gastrointestinal tract taken out. The stomach was cut open and gently blotted to remove mucus. The small intestine, 70–80 cm. long, was divided into three equal parts. Since the small intestine was almost empty, it was not opened. The caecum and the large intestine were cut open, the contents removed and the mucosa was blotted. All tissues were weighed, and homogenized for 2 min. in an Ultra-Turrax homogenizer with 4 ml. of 0·9% sodium chloride/g. of tissue. The tube was chilled with crushed ice before and during homogenization. After the homogenates had been centrifuged at 2000*g* for 10 min. the opalescent supernatants were used for the determinations of carbohydrase activity and protein, in order to evaluate the distribution of the enzymes between the different organs. In some experiments a sample of the caecal contents was similarly homogenized.

Pancreatic juice. To collect pancreatic juice the lower end of the common bile–pancreatic duct was cannulated during ether narcosis with a polyethylene tube. To avoid contamination with bile, this was removed through another cannula situated in the common bile duct near the liver. The animal was then placed in a small cage, allowing little movement, and the pancreatic juice was collected during 24 hr. in tubes chilled with solid carbon dioxide.

Subcellular fractions of mucosal homogenates. The small intestine was cut open and the mucosa removed with a piece of glass. From each rat 2–2·5 g. of small-intestinal mucosa was obtained. It was homogenized with 4 ml. of ice-cold 0·25 M-sucrose solution/g. in a homogenizer of the Potter & Elvehjem (1936) type with a glass pestle. During homogenization and all subsequent steps the solution was chilled to prevent hydrolysis of the sucrose.

After removal of the nuclei and cell debris by centrifuging at 1000*g* for 10 min. in an International Refrigerated centrifuge, differential centrifuging was performed in a Spinco preparative ultracentrifuge (Hogeboom, 1953), with rotor no. 40. A fraction was first sedimented at 7000*g* for 20 min. ('mitochondria'), then resuspended in 0·25 M-sucrose to give the original volume of the homogenate, and again sedimented at 7000*g* for 20 min. The combined supernatants were centrifuged at 100 000*g* for 45 min. giving a sediment ('microsomes') and a particle-free supernatant. Before analysis for carbohydrase activities the

sucrose was removed by dialysis against running tap water at 11° for 15–20 hr.

Solubilized mucosal enzymes. Small-intestinal mucosa was homogenized for 2 min. in the Ultra-Turrax homogeniser with 1 ml. of 0·2 M-sodium phosphate buffer (pH 7·0)/g. of tissue. During the homogenization the tube was chilled with ice. Then 1 mg. of crystalline trypsin was added/ml. of homogenate, and the tube was placed in a water bath at 37° for 2 hr. After this incubation the homogenate was chilled with crushed ice and the proteins were then precipitated by the addition of 3 vol. of 95% (v/v) ethanol, previously chilled to −16°. After centrifuging in an International Refrigerated centrifuge at 1000*g* for 10 min. the supernatant was discarded, and the sediment was taken up in water to give twice the volume of the original homogenate. The solution was centrifuged at 1000*g* for 10 min., and the sediment was discarded. The supernatant, which contains the solubilised carbohydrases, was dialysed for 16–24 hr. against water at 5°. It was stored at −16°.

Enzyme-fractionation methods

Anion-exchange chromatography. Diethylaminoethylcellulose (DEAE-cellulose) was prepared from Solka Floc cellulose powder SW 40 A by the method of Peterson & Sober (1956). The product contained 0·8 m-equiv. of titratable alkaline groups/g. dry wt., titrated in 0·5 M-sodium chloride (Peterson & Sober, 1956). By ethylation as described by Porath (1957) the diethylaminoethylcellulose was converted into triethylaminoethylcellulose (TEAE-cellulose). This ion-exchanger was stored and used in its bromide form. For preparation of the columns the ion-exchanger was first stirred with 100 ml. of 0·01 M-phosphate buffer/g. for 30 min. The column was washed with 500–1000 ml. of the same buffer/g. before application of the protein.

Heat inactivation. Heat inactivation of the enzyme preparations was performed as described by Dahlqvist (1959*a*).

RESULTS

Distribution of carbohydrases in the digestive tract

Tissue homogenates. Homogenates of all parts of the small intestine of starved rats had considerable dextranase activity, whereas homogenates of the stomach, caecum and colon were poor in dextranase (Table 1). The dextranase activity was somewhat higher in the proximal two-thirds of the small intestine than in its distal third. Amylase activity, in contrast, was present in considerable amount in all parts of the digestive tract. The disaccharidases, like the dextranase, were mainly localized in the small intestine. They were, however, not uniformly distributed along the small intestine. The invertase and isomaltase activities had a distribution similar to that of the dextranase, but the trehalase was considerably more concentrated in the proximal part of the small intestine, and the maltase activity was about equal in all three parts (Table 1). Nine rats investigated all showed essentially the same distribution of the carbohydrase activities.

Pancreatic juice. Rat pancreatic juice containing 40 mg. of protein/ml. had very powerful amylase (10 000 units/ml.), weak maltase (1–1·5 units/ml.), and no detectable dextranase, invertase, isomaltase or trehalase activity (less than 0·02 unit/ml.).

Caecal contents. Homogenates of the caecal contents of starved rats contained only low activities of the carbohydrases studied (Table 1).

Intracellular localization of carbohydrases

The major part of the dextranase, invertase and isomaltase activities of mucosal homogenates were recovered in the particulate fractions on differential centrifuging (Table 2). The three activities were distributed in a parallel manner, with the highest activity (units/mg. of protein) in the 100 000*g* fraction.

Solubilization and fractionation of enzymes

After digestion of homogenates of the mucosa of the small intestine with trypsin and purification by ethanol precipitation, 75% of the dextranase activity was recovered in soluble form together with the other carbohydrases. This preparation was used for a study of the separation of the enzymes by anion-exchange chromatography and heat inactivation. Trehalase activity was not followed in these experiments.

Anion-exchange chromatography. When the solubilized enzyme preparations were chromatographed on TEAE-cellulose, several peaks were obtained (Fig. 1). A column (1·0 cm. × 5·5 cm.) containing

Table 1. *Protein content and some carbohydrase activities of different parts of the gastrointestinal tract of a starved rat, measured* in vitro *in tissue homogenates*

Experimental details are given in the text.

Tissue	Weight of tissue (g.)	Protein (mg.)	Dextranase (units)	Amylase (units)	Invertase (units)	Maltase (units)	Isomaltase (units)	Trehalase (units)
Stomach	1·4	95	0·16	148	0·26	2·0	0·23	0·16
Small intestine								
Proximal third	2·4	273	4·7	271	8·0	30·1	9·1	10·0
Middle third	1·9	204	4·9	226	7·9	34·5	9·1	3·5
Distal third	2·1	179	2·8	850	3·2	34·4	6·3	0·37
Caecum	1·1	83	0·15	7·6	0·25	0·41	0·33	0·00
Colon	1·7	134	0·16	165	0·16	0·35	0·16	0·04
Caecal contents	1·7	51	0·12	3·3	0·42	0·06	0·19	0·03

Table 2. *Intracellular distribution of the dextranase, invertase and isomaltase activities in subcellular fractions from 1 ml. of a homogenate of rat-small-intestinal mucosa*

Experimental details are given in the text.

Fraction	Protein (mg.)	Dextranase (unit)	Invertase (unit)	Isomaltase (unit)
7 000*g* sediment ('mitochondria')	1·0	0·14	0·34	0·26
100 000*g* sediment ('microsomes')	0·9	0·19	0·52	0·30
Particle-free supernatant	1·8	0·13	0·44	0·19

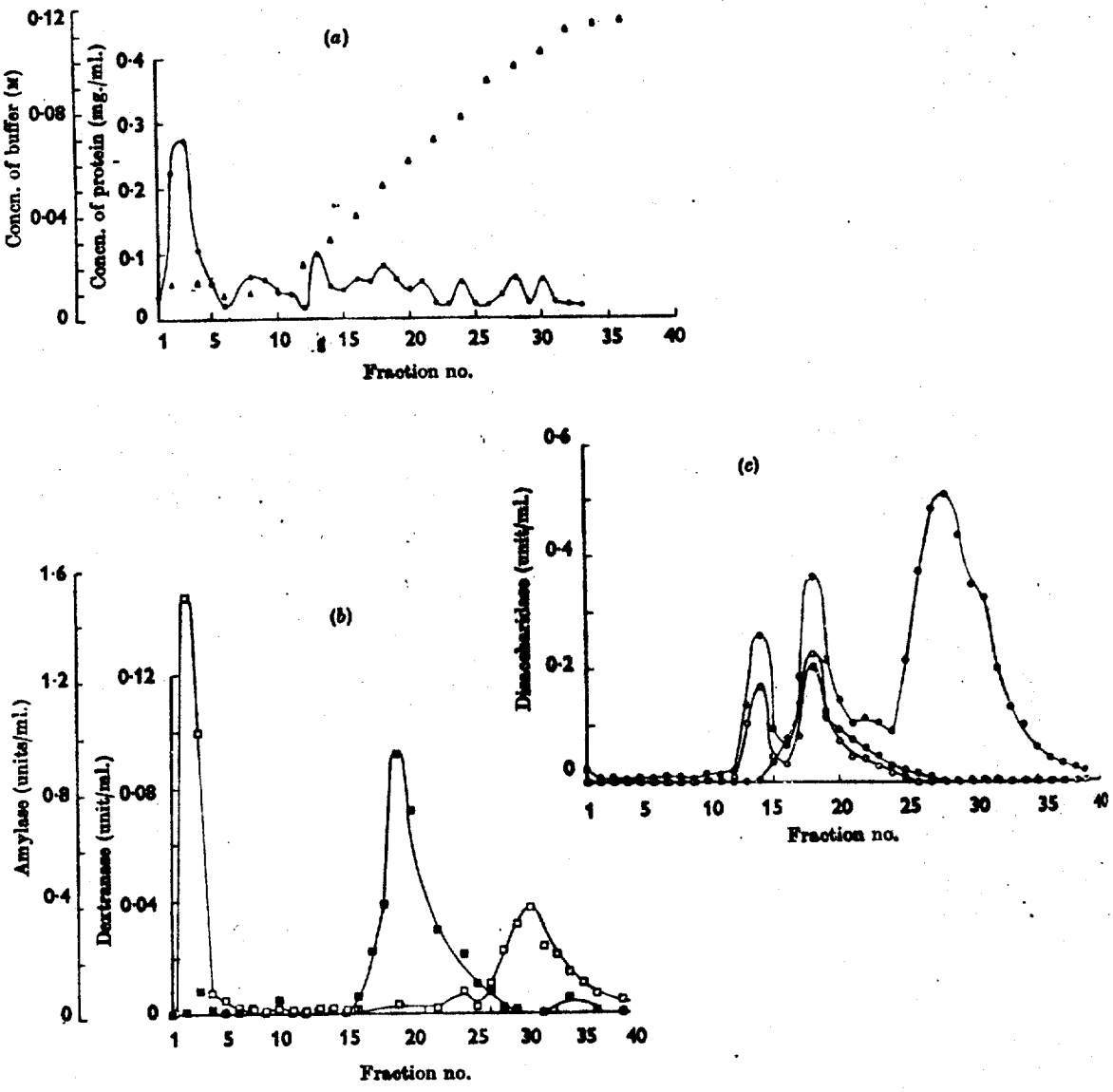

Fig. 1. Gradient-elution chromatography of rat-intestinal carbohydrases on TEAE-cellulose. Experimental details are given in the text. (*a*): △, Buffer concentration; ●, protein concentration. (*b*): □, Amylase; ■, dextranase. (*c*): ●, Maltase; ⊙, isomaltase; ○, invertase.

0·5 g. of TEAE-cellulose was used. A solubilized intestinal-mucosal preparation containing 6 mg. of protein, 1·8 units of dextranase, 16·2 units of amylase, 3·7 units of invertase, 17·1 units of maltase and 3·4 units of isomaltase, dissolved in 3·5 ml. of 0·01 M-phosphate buffer, pH 6·0, was applied to the column. The elution was performed at 20° with phosphate buffer, pH 6·0, of continuously increasing molarity, with a flow rate of 0·05–0·10 ml./min. Each fraction collected had a volume of 2·6 ml. In the effluent 86 % of the protein and 55–80 % of the carbohydrase activities were recovered.

The dextranase activity appeared as a single peak. The amylase activity was recovered in two peaks, both of which were separated from the dextranase (Fig. 1). The disaccharidase activities formed three different peaks. All of these three peaks contained maltase, two of them invertase and only one isomaltase. One disaccharidase peak appeared in the same fractions as the dextranase; this peak contained all of the isomaltase, about two-thirds of the invertase and one-fifth of the maltase activity recovered in the effluent.

Heat inactivation. The course of the heat inactivation of the carbohydrase activities of solubilized intestinal-mucosal preparations was followed at temperatures varying between 45° and 60° and pH values between 5·0 and 8·0. The enzyme solution had previously been dialysed against the appropriate buffer (0·01 M-sodium acetate and 0·01 M-sodium phosphate buffers) for 20 hr. at 4°.

The dextranase, invertase and isomaltase activities under the conditions tested were all inactivated, following the course of a first-order reaction, i.e. when the logarithm of the amount of activity remaining was plotted against time a straight line was obtained (Fig. 2). The heterogeneity of the rat-intestinal invertase observed chromatographically thus could not be demonstrated in the heat-inactivation experiments. Under all conditions the inactivation of the dextranase and isomaltase activities ran closely parallel, but the invertase activity is exerted by an enzyme with a clearly different sensitivity to heat. At pH 5–7 the invertase was inactivated more rapidly than the dextranase and isomaltase activities, but at pH 8 the last two activities were more sensitive to heat than was the invertase. After heat-treatment at 50° for 1 hr. at pH 6·0 the invertase was completely inactivated, but 60 % of the isomaltase and dextranase activities remained.

The relation of the maltase activity to the other disaccharidase activities could not be evaluated from the heat-inactivation experiments, owing to the complicated course of the inactivation of maltase (Fig. 2). This course reflects the chromatographic heterogeneity of the maltase activity.

In some experiments the course of the heat inactivation of the amylase activity was also followed. The shape of the curve indicated the presence of two amylases with different sensitivities to heat.

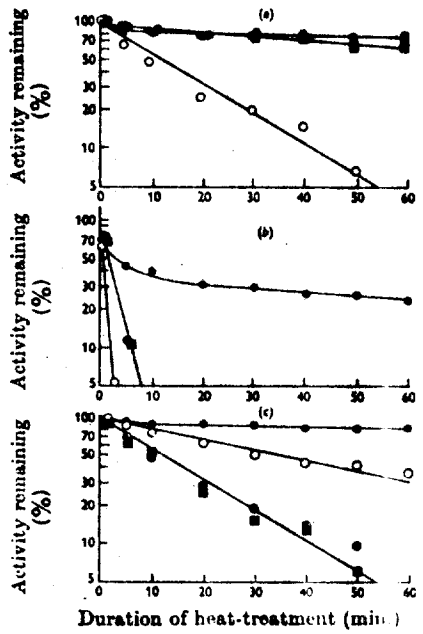

Fig. 2. Heat inactivation of the dextranase, invertase, maltase and isomaltase activities of solubilized rat-intestinal carbohydrase preparation (*a*) at 50° and at pH 6·0, (*b*) at 60° and at pH 6·0, and (*c*) at 45° and at pH 8·0. The buffer was 0·01 M-sodium phosphate. ○, Invertase; ●, maltase; ⊙, isomaltase; ■, dextranase.

DISCUSSION

In both rat and man the oral administration of dextran causes a rapid increase in blood sugar (Bloom & Wilhelmi, 1952), which has been assumed to mean that the polysaccharide is hydrolysed by a genuine intestinal enzyme and not solely by bacterial action (Bloom & Wilhelmi, 1952; Fischer & Stein, 1960). In agreement with this, dextranase was in the present study found to be localized mainly in the small intestine. The large intestine and the caecal contents had very little dextranase activity, which seems to rule out the possibility that the enzyme is of bacterial origin. The enzyme is probably formed in the small-intestinal mucosa, since the pancreatic juice did not contain dextranase. In homogenates of the small-intestinal mucosa the dextranase, like the disaccharidases, was mainly present in insoluble form, but could be solubilized by digestion with trypsin without appreciable loss of activity. The distribution of the dextranase activity in the

gastrointestinal tract is quite different from that of amylase. That amylase is distributed in all parts of the gastrointestinal tract is in accordance with the findings of McGeachin & Ford (1959). The separation of the dextranase and amylase activities by anion-exchange chromatography also clearly shows that they are exerted by different enzymes. The separation of the amylase in two different peaks does not seem to have been observed previously. The origin and specificity of these two amylases is as yet unknown.

Location in the small-intestinal mucosa is similar for the dextranase and the disaccharidases. The activities were not uniformly distributed along the small intestine. The trehalase activity was highest in the proximal third of the small intestine; the dextranase, invertase and isomaltase activities were strong in the whole small intestine, but highest in its proximal two-thirds; the maltase activity was about equal in all parts of the small intestine. The unequal distribution of these activities has previously been observed in the pig small intestine (Dahlqvist, 1961*a*).

The fractionation of the intestinal disaccharidases into several enzymes is in accordance with observations in pig (Dahlqvist, 1959*a*, *b*, 1960*a*) and man (Dahlqvist, 1962). The rat-intestinal invertase was fractionated into two components by anion-exchange chromatography, whereas in pig and man no heterogeneity of the intestinal invertase has been revealed. Although the different species hitherto investigated have a roughly similar set of intestinal disaccharidases, marked species differences may exist. Differences in specificity between the disaccharidases of the pig and man have been noted (Dahlqvist, 1962).

The fractionation methods separated the dextranase activity completely from the amylase and invertase activities, and also from the main part of the maltase activity. The trehalase activity was not studied in the fractionation experiments, but the distribution of the dextranase and trehalase activities in the small intestine shows that they belong to separate enzymes. In all the experiments, however, the dextranase and isomaltase activities closely followed each other.

These activities also were distributed in a parallel way in the digestive tract with a constant isomaltase:dextranase activity ratio of 2·0, which strongly suggests that the rat-intestinal dextranase and isomaltase are a single enzyme.

SUMMARY

1. The intestinal dextranase is confined to the small-intestinal mucosa. Like the disaccharidases it is chiefly particulate in mucosal homogenates, but can be solubilized with trypsin without appreciable loss of activity.

2. The distribution of the dextranase and the results of fractionation (by anion-exchange chromatography and heat inactivation) demonstrate that the dextranase is distinct from amylase, invertase, trehalase and at least the major part of the maltase activity.

3. Isomaltase, in contrast, was indistinguishable from the dextranase with a constant isomaltase:dextranase activity ratio of 2·0. It is suggested that these two activities are exerted by the same enzyme.

4. The amylase activity of mucosal preparations was found to belong to two different enzymes. The disaccharidase activities were exerted by a mixture of enzymes, roughly similar to those previously recognized in preparations from pig- and human small-intestinal mucosa, but showing a specific difference in containing two invertases.

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Experimental Studies of Factors Influencing Hepatic Metastases
VI. Rheologic Alterations[1]

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Summary

The incidence and size of hepatic metastases following intrasplenic injection of known numbers of Walker carcinoma cells were increased after administration of low, medium, and high molecular weight dextrans. The dextrans were not hepatotoxic, and their effect on tumor growth appeared to be unrelated to alterations they induced in viscosity of the blood or hematocrit. Simple suspension of tumor cell inocula in low molecular weight dextran also resulted in augmentation of tumor growth, suggesting that the dextrans may exert more direct, albeit at present unclear, growth-promoting effect on tumors.

During the course of these studies a consistent and roughly quantitative relationship was observed between tumor growth and increase in circulating blood volume resulting from the administration of low molecular weight dextran or other modalities such as plasma or saline infusions. Possible mechanisms accounting for this effect are considered.

The failure to observe alteration in blood viscosity following liver injury, a modality consistently noted to augment tumor growth in the experimental model utilized for evaluating metastases, makes it highly unlikely that the effect of liver injury on tumor growth is related to the rheologic alteration that has been observed to follow tissue injury in man and dog.

The results of this study further suggest that utilization of agents that expand blood volume during neoplastic surgery might be injudicious.

Introduction

While extensive information relative to the biologic aspects of tumor metastases has accumulated (9, 11, 15, 16, 35, 45, 48, 51), much of the process remains a mystery. In particular, little is known of the precise mechanism whereby a circulating tumor cell becomes arrested in an intravascular position. Greene and Harvey (27) suggested that vascular "receptivity" to tumor cells is of importance in this process and that cells become adherent to the vascular endothelium in the fashion of leukocytes at the site of inflammation. Since (*a*) thrombi occur around arrested tumor cells (49, 51), (*b*) animal and human cancer cells may be rich in thromboplastin (5, 8, 30, 33, 34, 37), (*c*) hypercoagulability with its associated reduction in fibrinolytic activity increases the number of metastases (51), and (*d*) heparin (13, 52) and fibrinolysin (8, 50) decrease the incidence of metastases, it has also been conjectured that the coagulation mechanism may play a vital role in the initial adherence of cancer cells and their subsequent development into overt metastases. Whatever the mechanism(s) involved, adhesion of tumor cells and succeeding metastases occur most frequently in the microvasculature, i.e., the capillaries (46, 50).

In recent years there has been a remarkable revival of interest in the rheologic events that occur in this portion of the vascular compartment, in both health and disease. The impetus for such studies has come primarily from the investigation of Knisely *et al.* (31), which attracted attention to the importance of cell aggregation or "blood sludging" in the microcirculation, and from the extensive investigation of Gelin *et al.* (25) following the advent of dextran. Information relative to the rheology of blood in the microvasculature, to the use of low molecular weight dextran to alter capillary flow, and to technics employed for such studies has recently been reviewed (10, 24, 40, 47).

Knowledge relative to the rheology of blood in the microvasculature and the development of tumor metastasis, in spite of its seeming importance, is not available. Investigations, the results of which are reported here, were carried out with the purpose of obtaining such information consequent to a variety of hematorheologic alterations.

Methods

Sprague-Dawley female rats weighing 180–200 gm were used in all experiments. They were housed in individual cages and were permitted water and a standard laboratory chow diet *ad libitum*. Hematocrit determinations were made on blood from the tail of all animals with the Drummond microhematocrit and double-oxalate tubes. Only those samples having values ±2 points from the average of 42 were used. When heparinized blood was employed, untreated tubes were used.

Viscosity of blood was measured immediately after withdrawal in the Brookfield Synchro-Lectric viscometer (Brookfield Engineering Laboratories, Stoughton, Massachusetts) at 37°C and is expressed as centipoise. Viscosity was determined over the entire range of the instrument, 60–0.3 rpm, and only values obtained from 6 to 60 rpm are reported. Within this range the instrument is accurate to within less than ±1 centipoise. To ensure the representative nature of the blood sample employed for viscosity determination, a comparison was made of the viscosities of blood samples taken simultaneously from the left heart and the femoral and portal veins. Results were identical from the 3 sites.

SGP-T[2] was determined on blood drawn by cardiac puncture and is expressed in Sigma-Frankel units (41).

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[2] The following abbreviations are used: SGP-T, serum glutamic pyruvic transaminase; RISA ^{131}I, radioactive iodinated serum albumin; LMW, low molecular weight; MMW, medium molecular weight; HMW, high molecular weight; RE, reticuloendothelial.

Phagocytic activity of the reticuloendothelial system following saline and low molecular weight dextran was assessed by the rate of carbon clearance as described by Biozzi *et al.* (3) and as previously reported from our laboratory (20, 21). The rate of clearance of carbon from the blood or the phagocytic index is expressed as a constant, K. The greater the K, the more rapid the carbon clearance. After this value has been corrected for body, liver, and spleen weight, it is expressed as α, the corrected phagocytic index.

Total blood volume was estimated by determining the dilution of RISA^{131}I (Abbott Laboratories, North Chicago, Illinois) in a 10-min postinjection sample of 1 ml of blood.

The Walker carcinoma used in these experiments has been propagated in this laboratory for the past 7 years. The method of preparation of tumor cell suspensions and the technic of injection have been described in detail (18). An equal number of animals from each group within an experiment received the same tumor cell suspension.

At sacrifice 14 days after tumor cell injection all animals were examined for liver metastases. Tumors, when present, were arbitrarily graded according to number and size. Small, occasional nodules were considered +1; larger nodules scattered throughout all lobes, +2; and almost complete replacement of the liver together with bloody peritoneal fluid, +3.

At sacrifice, complete autopsies were performed. Blocks of heart, lung, liver, spleen, adrenals, and kidney were fixed in absolute alcohol, further dehydrated with petroleum ether, and imbedded in paraffin. Sections were stained with hematoxylin and eosin, as well as according to the periodic acid-Schiff technic. The latter was also utilized in formalin-fixed tissues with and without antecedent diastase treatment. The demonstration of dextran within tissues was accomplished by comparing duplicate sections stained with aqueous and alcoholic periodic acid in the periodic acid-Schiff sequence according to the method of Mowry and Millican (36). Dextran, being water soluble, is removed in sections treated with the aqueous agent, but it is preserved in sections in which water is avoided. The utilization of diastase eliminates glycogen, which might interfere with the identification of dextran.

Experimental Design

EXPERIMENT 1. EFFECT OF ALTERATION OF HEMATOCRIT AND BLOOD VISCOSITY ON HEPATIC METASTASES. With the use of light open drop ether anesthesia, polyethylene catheters (PE 50) were anchored in the femoral veins of rats in all groups except untouched controls (Group A). Forty % of the calculated blood volume was withdrawn slowly over 5–7 min, and an equal volume of the fluid to be evaluated was reinfused. This procedure was repeated a 2nd time, so that 60% of the original plasma volume or 60% of the red cell mass, or both, were replaced. In sham controls (Group B) the animal's own blood was reinjected. Heparinized syringes were utilized for injections in both experimental and sham control rats.

In order to decrease the hematocrit (Group C), blood was replaced with the following cell-free fluids: (*a*) rat plasma; (*b*) LMW dextran (Rheomacrodex, Pharmacia Laboratories, Uppsala, Sweden), molecular weight 40,000 10% w/v in normal saline; (*c*) MMW dextran (American Dextran, Abbott Laboratories), molecular weight 75,000, 6% w/v in normal saline, and (*d*) HMW dextran (Dextraven, Benger Laboratories, Ltd., Holmes Chapel, Cheshire, England), molecular weight 150,000, 6% w/v in normal saline.

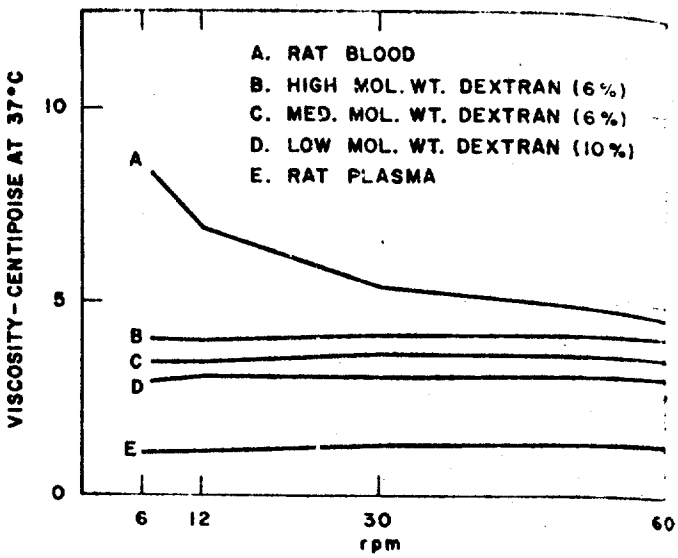

CHART 1. Viscosity of various solutions employed for replacement of rat blood.

The viscosities of the various fluids used for replacement were determined (Chart 1). Rat plasma had the lowest viscosity and HMW dextran the greatest. All were considerably less viscous than rat blood.

To increase the hematocrit (Group D), red cells from donor rats were separated from the plasma by centrifugation, washed with normal saline and resuspended in sufficient saline to produce a hematocrit of 80 in the solution to be infused.

In Group E red cells separated from the plasma were washed and resuspended in low, medium, or high molecular weight dextran solutions to reproduce the normal hematocrit. All animals were injected intraportally with 5000 Walker tumor cells 24 hr after blood replacement.

A series of identically managed animals, not injected with tumor, were utilized for hematocrit, viscosity, blood volume, and transaminase determinations at varying times following blood replacement.

EXPERIMENT 2. EFFECT OF PROLONGED INFUSION OF LMW DEXTRAN OR SALINE ON HEPATIC METASTASES. Polyethylene catheters were anchored in the jugular veins of all animals. Rats (including controls) were placed in restraining cages with appropriate harness so that they had a fair amount of mobility but were unable to destroy the catheters. With a constant rate infusion pump, rats were given 8 ml/24 hr of either saline or LMW dextran. Infusion was begun immediately after tumor cell injection and was continued for 24 and 48 hr (Groups A and B respectively). In Group C tumor cells were injected at the midpoint of the 48-hr infusion. At the termination of infusion catheters were occluded and animals were returned to individual cages. The number of tumor cells inoculated and times of sacrifice of animals were similar to those in Experiment 1.

Identical groups of animals were subjected to hematocrit,

...osity, blood volume, and transaminase determinations 24 and ... hr after infusion.

EXPERIMENT 3. EFFECT ON METASTASIS OF INJECTION OF LMW ...XTRAN OR SALINE PRIOR TO AND AFTER TUMOR CELL INOCULA...N. Jugular catheters were inserted into all animals 24 hr prior ...tumor cell inoculation. Animals in Group A served as unin... tumor-inoculated controls. Rats in Group B were given ...ctions via the jugular catheters 1, 6, or 24 hr before tumor cell ...ion with either 2 ml of normal saline or 2 ml of LMW ...ran. In Group C the injections of saline and dextran were ...ed out 1, 6, or 24 hr after tumor cell inoculation. All animals ...ived 5000 tumor cells. They were sacrificed 14 days later and ...mined for tumor.

Rats prepared in identical fashion were used for determination ...hematocrit, blood volume, and blood viscosity.

EXPERIMENT 4. EFFECT OF SIMULTANEOUS INJECTION OF TUMOR ...LS IN LMW DEXTRAN. The solid tumor was gently pressed ...ough a cytosieve, and a portion was suspended in either saline ...LMW dextran. Proper dilutions were made and 5000 tumor ...s in either 0.5 ml of saline or dextran were inoculated intra...tally. Sacrifice time was similar to that for the other experi...nts.

EXPERIMENT 5. EFFECT OF HEPATIC TRAUMA ON BLOOD VISCOS...TY—RELATION TO METASTASES. Animals were divided into 3 ...oups: (*a*) 10 animals served as unoperated controls for vis...sity and hematocrit determinations; (*b*) 21 animals were ...bjected, while under ether anesthesia, to simple laparotomy ...hout touching the liver; and (*c*) 21 rats were laparotomized ...t subjected to liver manipulation. Blood from animals in the ...er groups was drawn 6 and 24 hr later for determination of ...osity and hematocrit.

Results

Effect of Alteration of Hematocrit and Blood Viscosity on Hepatic Metastasis (Table 1)

Hematocrits, viscosities, and blood volumes obtained from 120 ...al rats (Group A) served as control values. These animals had an average hematocrit of 41.2 ± 2.4, a viscosity of 8.3 ± 2.1 centipoise at 6 rpm, and a blood volume of 12.0 ± 1.3 ml (6.4 ± 0.4% of their body weight). When similar animals were injected intraportally with 5000 tumor cells, 44% demonstrated hepatic metastases at sacrifice; 19% of the tumors were classified as +2 or +3.

Sham-treated control animals (Group B) demonstrated an insignificant decrease in viscosity and hematocrit 24, 48, and 96 hr and 8 days after manipulation (Charts 2 and 3). Likewise, the blood volume after 24 hr was only slightly increased (12.9 ± 1.0 ml or 6.9 ± 0.1% of body weight). No difference in degree or incidence of metastases from that found in normal controls (Group A) was encountered.

When a part of the red cell mass was replaced with plasma, or with low, medium, or high molecular weight dextran (Group C), a profound reduction in both viscosity and hematocrit was produced, and it persisted for as long as 8 days (Charts 2 and 3). Twenty-four hr after LMW dextran replacement, the blood volume was 9.0 ± 1.8% of body weight and following plasma substitution, 9.1 ± 1.1% of body weight. Animals injected with tumor cells at the peak of such an alteration demonstrated a striking increase in the incidence and degree of hepatic metastases. This particularly was evident in the dextran-treated animals. The employment of HMW dextran resulted in the greatest augmentation and of plasma, in the least. Following MMW and HMW dextran, the blood volumes were slightly increased (10.9 ± 11.1% and 11.7 ± 6.0%).

Replacing plasma with packed red cells (Group D) resulted in a sustained increase in viscosity and hematocrit (Charts 2 and 3) and a significant increase in blood volume. Animals inoculated with tumor demonstrated the same findings as those in which viscosity and hematocrit had been lowered—an increased incidence and degree of liver metastases.

When plasma alone was replaced by low, medium, or high molecular weight dextran (Group E) the hematocrit was essentially unaltered. The viscosity was only slightly lowered by MMW and HMW dextran. Incidence of metastases and the

TABLE 1

EFFECT OF ALTERATION OF HEMATOCRIT, BLOOD VISCOSITY, AND BLOOD VOLUME ON DEVELOPMENT OF HEPATIC METASTASES

GROUP	AT TIME OF TUMOR INJECTION			BLOOD VOLUME			HEPATIC METASTASES		
	No. of rats	Hematocrit	Viscosity[a]	ml	% body wt.	Increase (%)	No. of rats	% +	% 2-3+
Normal control	120	41.2 ± 2.4	8.3 ± 2.1	12.0 ± 1.3	6.4 ± 0.4		299	44	19
Sham control	24	38.8 ± 1.8	7.4 ± 1.3	12.9 ± 1.0	6.9 ± 0.1	7.5 ± 2.1	114	40	13
Low hematocrit									
1. Plasma	12	13.2 ± 0.4	2.4 ± 0.7	15.3 ± 2.6	9.1 ± 1.1	41.9 ± 17.5	65	65	29
2. LMW[b] dextran	12	17.6 ± 1.3	2.6 ± 0.6	15.6 ± 2.6	9.0 ± 1.8	40.4 ± 27.8	46	87	77
3. MMW dextran	12	15.7 ± 0.6	2.5 ± 0.2	13.9 ± 1.2	7.1 ± 0.7	10.9 ± 11.1	38	84	56
4. HMW dextran	12	13.3 ± 2.4	2.1 ± 0.5	13.7 ± 0.7	7.1 ± 0.4	11.7 ± 6.0	21	95	90
High hematocrit	12	55.8 ± 1.7	13.3 ± 2.4	16.0 ± 0.5	8.0 ± 0.4	25.3 ± 6.2	46	63	48
Normal hematocrit									
1. LMW dextran	12	42.5 ± 1.4	8.0 ± 1.1	15.1 ± 0.7	7.8 ± 0.4	21.3 ± 6.2	42	69	55
2. MMW dextran	12	38.5 ± 1.0	6.9 ± 0.9	15.9 ± 1.4	8.2 ± 0.7	27.3 ± 10.9	43	81	66
3. HMW dextran	12	41.4 ± 1.3	5.9 ± 1.0	16.4 ± 1.1	8.5 ± 0.5	33.1 ± 7.9	36	80	69

[a] Centipoise (37°C) at 6 rpm.
[b] LMW, MMW, and HMW, low, medium, and high molecular weight, respectively.

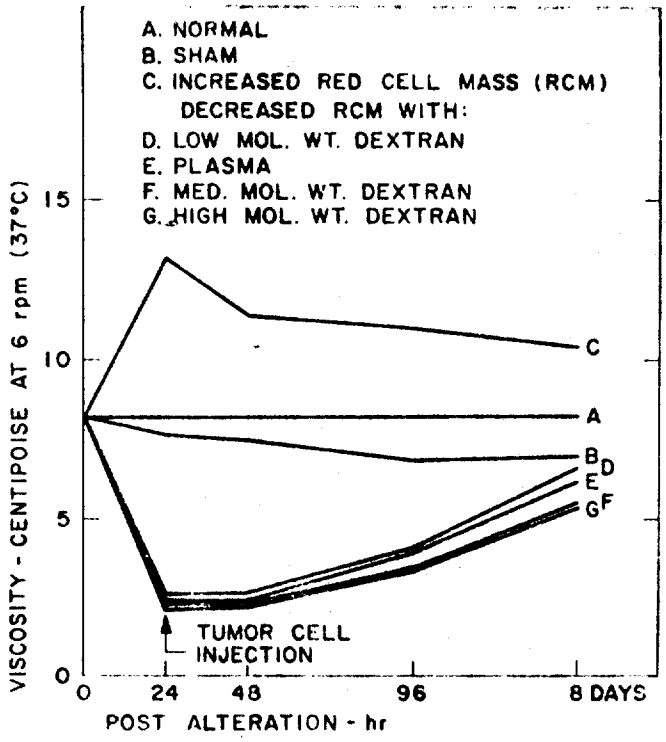

CHART 2. Effect of alteration of red cell mass on blood viscosity.

degree of tumor growth were greatly increased by all 3. Significant increases in blood volume followed the employment of all.

To determine whether such alterations of the blood might not result in tissue damage that might be reflected in changes in SGP-T, this parameter was determined 24 and 48 hr following the experimental manipulations (Table 2). At the earlier time, only groups with a lowered hematocrit that received low and high molecular weight dextrans had a significant elevation of this value. After 48 hr, in addition to those groups, the groups receiving plasma and HMW dextran (Groups C-1, C-4) had an increased SGP-T. Increasing the viscosity and hematocrit (Group D) or substituting the dextrans for plasma (Group E) had no effect on this parameter.

Effect of Prolonged Infusion on LMW Dextran or Saline on Hepatic Metastases (Table 3)

The infusion over 24 hr of an amount of LMW dextran equivalent to more than 50% of the animal's blood volume only slightly lowered blood viscosity (Chart 1), reduced the hematocrit from 45.6 ± 0.6 to 40.6 ± 2.4, and increased circulating blood volume 18.2 ± 12.8%. Administration of an equivalent amount of saline resulted in a greater reduction in hematocrit and viscosity but in not as great an increase in blood volume.

Continuation of the infusion of LMW dextran or saline for 48 hr so that animals received an amount of fluid almost equivalent to their initial blood volumes resulted in no significant change in hematocrit. An increase in viscosity (Chart 5) and in blood volume occurred after LMW dextran. Saline infusion produced no change in viscosity but did increase the blood volume by 7.2 ± 4.2%.

When animals were inoculated with tumor cells just prior to 24 or 48 hr of infusion with saline or LMW dextran (Groups A and B) or at the midpoint of a 48-hr perfusion (Group C), the incidence and degree of hepatic metastases observed in such animals were in all instances greater than in control, unperfused animals. Although saline infused for 24 hr increased the incidence of metastases, the augmentation was not as great as when LMW dextran was employed. Likewise, the magnitude of tumor growth was less. The incidence of metastases was the same when either saline or LMW dextran was infused for 48 hr, but tumor size was

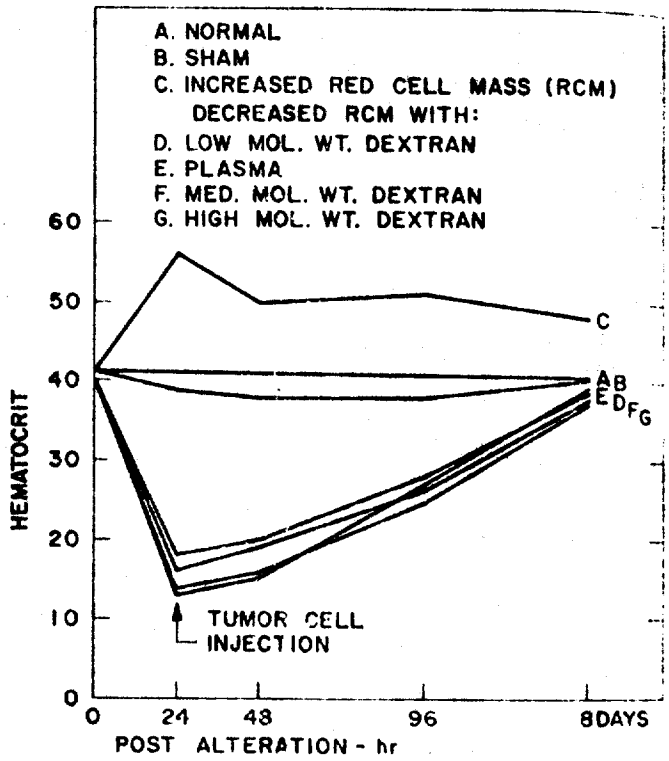

CHART 3. Effect of alteration of red cell mass on hematocrit.

TABLE 2

EFFECT OF ALTERATION OF HEMATOCRIT OR BLOOD VISCOSITY OR BOTH, ON SERUM TRANSAMINASE

GROUP	NO. OF RATS	SERUM GLUTAMIC PYRUVIC TRANSAMINASE	
		24 hr	48 hr
A. Normal controls	24	21 ± 8	
B. Sham controls	12	15 ± 8	18 ± 7
C. Low hematocrit			
1. Plasma	13	29 ± 14	35 ± 12
2. LMW dextran[b]	12	61 ± 50[a]	31 ± 18
3. MMW dextran	12	21 ± 4	29 ± 8
4. HMW dextran	12	48 ± 38[a]	44 ± 28
D. High hematocrit	12	17 ± 7	20 ± 13
E. Normal hematocrit			
1. LMW dextran	19	21 ± 19	17 ± 6
2. MMW dextran	17	28 ± 13	28 ± 13
3. HMW dextran	12	21 ± 13	20 ± 12

[a] $P < 0.01$ when compared to controls.

[b] LMW, MMW, and HMW, low medium, and high molecular weight respectively.

TABLE 3

EFFECT OF PROLONGED INFUSION[a] OF LMW DEXTRAN UPON DEVELOPMENT OF HEPATIC METASTASES

LENGTH OF INFUSION	GROUP	HEMATOCRIT[b]	BLOOD VOLUME[b]			HEPATIC METASTASES		
			ml	% body wt.	Increase (%)	No. of rats	% +	% 2-3+
A. 24 hr after tumor cell injection	Control (uninfused)	45.6 ± 0.6	14.4 ± 0.9	6.6 ± 0.3		19	37	29
	Saline	38.4 ± 1.5	15.0 ± 0.6	7.3 ± 0.6	10.0 ± 9.0	20	50	10
	LMW[c] dextran	40.6 ± 2.4	16.8 ± 1.3	7.8 ± 0.8	18.2 ± 12.8	20	70	71
B. 48 hr after tumor cell injection	Control (uninfused)	44.1 ± 1.6	11.7 ± 0.4	6.1 ± 0.1		20	40	25
	Saline	45.0 ± 1.8	12.8 ± 1.1	6.5 ± 0.3	7.2 ± 4.2	20	65	31
	LMW dextran	45.1 ± 1.8	13.3 ± 0.8	6.7 ± 0.2	9.3 ± 3.5	19	68	54
C. 24 hr before and 24 hr after tumor cell injection	Control (uninfused					20	35	29
	Saline					18	61	55
	LMW dextran					16	56	78

[a] 8 ml/24 hr.
[b] End of infusion.
[c] LMW, low molecular weight.

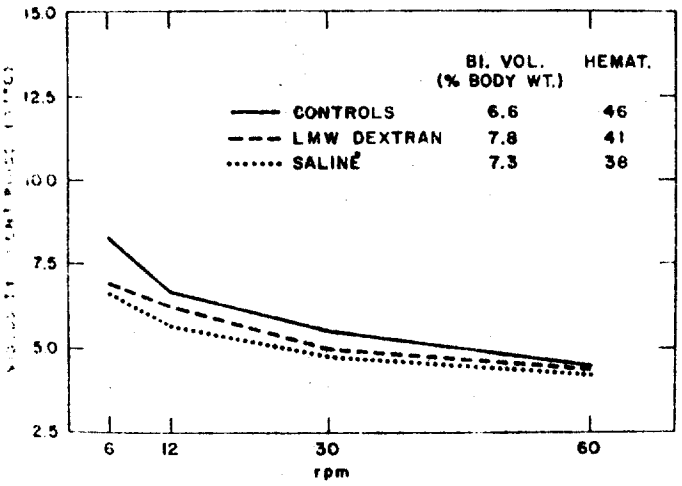

CHART 4. Viscosity immediately after a 24-hr infusion of saline and LMW dextran. Bl. VOL., blood volume; HEMAT., hematocrit.

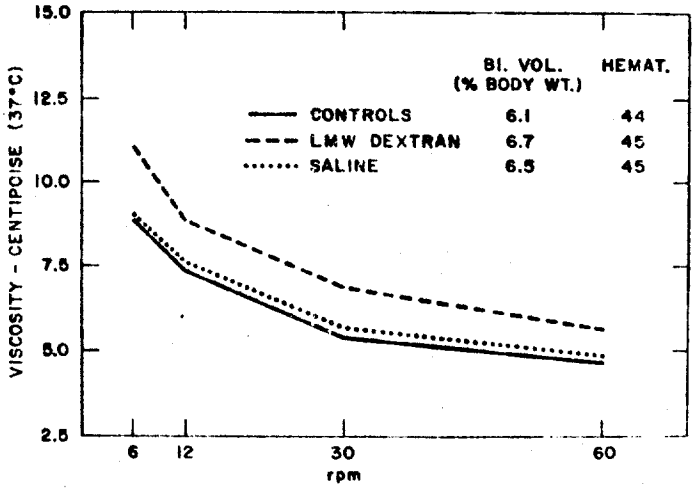

CHART 5. Viscosity immediately after a 48-hr infusion of saline and LMW dextran. Bl. VOL., blood volume; HEMAT., hematocrit.

...ter in animals receiving dextran. In all instances the degree ...tumor increase correlated directly with changes in blood ...ume.

...e prolonged infusion of saline or LMW dextran failed to ... the SGP-T. Values determined 24 and 48 hr following com... of infusion were not statistically different from those in ...ed animals (Table 4).

... of Injection of LMW Dextran or Saline Prior to and After ... Cell Inoculation on Metastasis (Table 5)

... hr following intravenous injection of 2 ml of normal saline ... hematocrit was found to be insignificantly altered. There was, however, an increase of 19.7% in the blood volume and a slight increase in viscosity, suggesting that the red cell mass had been increased. By 6 hr the hematocrit and viscosity had fallen and the blood volume had increased 36.7%. After 24 hr the blood volume was still significantly elevated. Following LMW dextran administration the changes were similar, but more pronounced. The blood volume had increased 34.5% 6 hr following infusion, at which time a notable decrease in blood viscosity was observed. Great variation from animal to animal in the response to infusion, particularly after 1 hr, was observed in these parameters. Injection of tumor cells 1, 6, or 24 hr after, or 1 hr before, LMW dextran infusion, resulted in an increase in both the

incidence and the magnitude of hepatic metastases. When the dextran was given 6 or 24 hr after tumor inoculation no effect on metastatic incidence was noted, but tumors present in the 6-hr group were larger than those in the controls.

Inoculation of tumor cells at any of the times after administration of saline resulted in an increase in metastases over the number observed in control animals; however, the difference was not as pronounced as after LMW dextran. When saline was administered after tumor cell inoculation, less of an effect was noted. As observed in the other experiments, a direct correlation of tumor growth with blood volume alterations was evident.

Twenty-four and 48 hr after saline administration the SGP-T values were 19 ± 3 and 23 ± 8 units, respectively. Following LMW dextran injection at these times, SGP-T's were 27 ± 7 and 25 ± 10 units—similar to the values of uninfused controls.

The carbon clearance in similarly treated animals 1, 6, and 24 hr after saline infusion was slightly decreased (Chart 6). One hr following LMW dextran injection the carbon clearance was markedly accelerated, but by 6 hr some evidence of delay in the function was noted.

TABLE—4

EFFECT OF PROLONGED INFUSION OF LMW DEXTRAN AND SALINE ON SERUM TRANSAMINASE

GROUP	LENGTH INFUSION (HR)	NO. OF RATS	SERUM GLUTAMIC PYRUVIC TRANSAMINASE	
			24 hr[a]	48 hr[a]
Experiment 1				
A. Sham (uninfused)		24	24 ± 8	
B. Saline	24	6	23 ± 2	25 ± 13
C. LMW[b] dextran	24	6	22 ± 5	20 ± 6
Experiment 2				
D. Saline	48	6	26 ± 8	23 ± 2
E. LMW dextran	48	7	26 ± 6	21 ± 6

[a] Postinfusion.
[b] LMW, low molecular weight.

Effect of Simultaneous Injection of Tumor Cells in LMW Dextran

When 20 rats were injected intraportally with a suspension of tumor cells in 0.5 ml of normal saline, 4, or 20%, demonstrable liver tumors at sacrifice, and none of the tumors was +2 or +3 in size. In another group of 20 rats injected with the same tumor cells suspended in LMW dextran, metastases developed in 55% of the animals, 9% of which had +2- or +3-sized tumors.

Effect of Hepatic Trauma on Blood Viscosity—Relation to Metastases (Table 6)

Blood viscosity and hematocrit determined 6 and 24 hr following simple laparotomy were not significantly different from unoperated control animals. Animals whose livers had been manipulated 24 hr previously, however, did demonstrate a significant reduction in blood viscosity and hematocrit.

APPEARANCE OF TUMOR. Various treatments with dextran and alteration in blood viscosity or blood volume failed to differentiate the histologic appearance of the Walker tumor from that noted in controls.

APPEARANCE OF DEXTRANS IN TISSUES. All dextrans employed could be identified in hepatic parenchymal and Kupffer cells, sinusoidal and perivascular macrophages in the lungs and heart.

TABLE 5

EFFECT OF INJECTION (2 ml) OF LMW DEXTRAN AND SALINE ON HEMATOCRIT, BLOOD VOLUME, VISCOSITY, AND HEPATIC METASTASES

GROUP	NO. OF RATS	HEMATOCRIT	BLOOD VOLUME			VISCOSITY[a]	HEPATIC METASTASES		
			ml	% body wt.	Increase (%)		No. of rats	% +	% 2-3+
A. Controls	12	40.0 ± 1.7	12.5 ± 1.3	6.4 ± 0.4	—	6.2 ± 1.2	59	30	0
B. After saline									
1 hr[b]	12	42.9 ± 3.3	14.9 ± 1.9	7.8 ± 0.6	19.7 ± 8.8	7.1 ± 1.3	30	60	17
6 hr	12	37.5 ± 3.9	17.1 ± 4.0	8.7 ± 1.9	36.7 ± 29.7	6.0 ± 1.0	29	59	12
24 hr	12	39.0 ± 4.1	14.3 ± 1.4	7.4 ± 0.7	16.1 ± 11.9	7.1 ± 1.0	29	48	14
C. After dextran									
1 hr	12	43.9 ± 9.2	15.7 ± 5.0	8.1 ± 2.6	27.1 ± 40.9	5.1 ± 1.8	28	86	92
6 hr	12	35.7 ± 2.5	17.0 ± 1.9	8.6 ± 0.9	34.5 ± 13.8	4.9 ± 1.0	22	91	60
24 hr	12	40.1 ± 3.3	14.3 ± 1.5	7.6 ± 0.6	19.5 ± 9.8	6.7 ± 1.8	26	61	50
D. Before saline									
1 hr							14	43	17
6 hr							26	23	33
24 hr							15	33	0
E. Before dextran									
1 hr							14	64	44
6 hr							22	36	55
24 hr							13	23	0

[a] Centipoise (37°C) at 6 rpm.
[b] Time of tumor cell inoculation relative to saline or dextran injection.

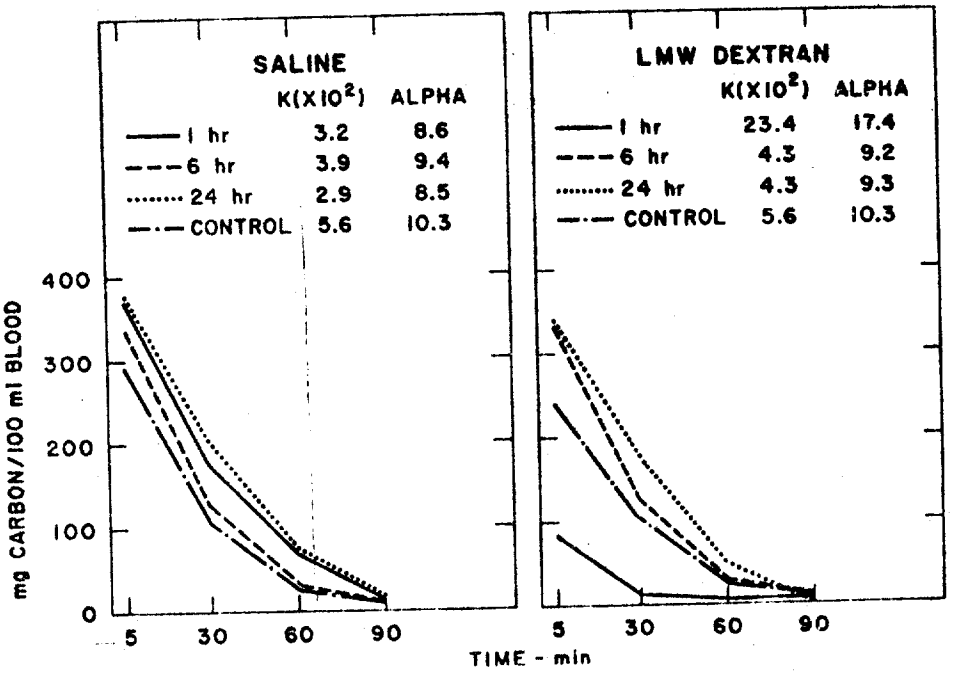

CHART 6. Carbon clearance 1, 6, and 24 hr following i.v. injection of saline or LMW dextran.

TABLE 6

EFFECT OF HEPATIC TRAUMA ON BLOOD VISCOSITY AND HEMATOCRIT

GROUP	TIME AFTER TRAUMA (HR)	NO. OF RATS	HEMATOCRIT	VISCOSITY AT 37°C (CENTIPOISE)			
				6 rpm	12 rpm	30 rpm	60 rpm
A. Unoperated controls		10	42 ± 1.1	6.3 ± 1.3	5.4 ± 0.9	4.2 ± 0.5	3.7 ± 0.4
B. Laparotomy	6	10	42 ± 2.7	6.6 ± 2.1	5.8 ± 1.7	4.3 ± 0.9	3.8 ± 0.6
	24	11	40 ± 2.2	6.7 ± 0.9	5.7 ± 0.7	4.6 ± 0.5	4.0 ± 0.3
C. Hepatic trauma	6	10	39 ± 3.5	6.1 ± 2.0	5.3 ± 1.5	4.0 ± 0.8	3.5 ± 0.5
	24	11	34 ± 1.3[a]	5.0 ± 0.9[a]	4.6 ± 0.7[a]	3.8 ± 0.5	3.5 ± 0.4

[a] Significantly different from controls.

ip macrophages in the spleen, sinusoidal cells of the adrenal and sinusoidal and tubular epithelial cells of the kidney. ght variations in the amount of dextran were observed in the ious experimental situations. However, it wasgenerally noted t the maximum quantity appeared in the locations cited n 24 and 96 hr after administration and that, except for occasional macrophage in the spleen, all tissues were devoid dextran by 8 days.

iscussion

The dextrans, particularly those of higher molecular weight, e been reported to interfere with a variety of coagulation fac- 6, 7, 32). A transient prolongation of bleeding time during period of plasma volume expansion has been noted following sion of LMW dextran to normal subjects (25). Thrombo- ic material from human tumors has been inhibited *in vitro* LMW dextran (49).

A reduction in blood viscosity with resultant increase in eral flow is reputed to occur following the administration LMW dextran. This is a consequence of the dilution of all elements on the blood (including fibrinogen) by a large amount of low-viscosity interstitial fluid, which is attracted into the plasma from across the capillary wall as a result of increased colloidal osmotic pressure (26). Also, LMW dextran reduces and reverses cell aggregation by the direct effect on the electrical charge of the cell surface (2) or, as proposed, by physically or chemically binding fibrinogen (55). While the higher molecular weight dextrans increase the aggregation of red cells and thus cause capillary emboli in parenchymatous organs (28, 29), dextrans with lower molecular weights reverse this phenomenon (44).

All of these factors, and others relative to the rheologic effects of the dextrans, might well influence the course of events in the lodgment and attachment of circulating tumors, about which most of our knowledge comes from the microcinematographic studies of Zeidman (54) and Wood (49, 51). The latter investigator has observed that within a matter of minutes following lodgment of tumor cells in capillaries the cells become surrounded by a microcoagulum of fibrin and platelets, and a microthrombus results. After a brief interval, the endothelium adjacent to the thrombus loses its normal appearance, and leukocytes accumu-

late at those endothelial sites. These cells traverse the minute defects in the vessel wall and are followed by cancer cells, which rapidly migrate into the perivascular tissues. Such cells are reported either to rapidly undergo mitotic division and grow as an overt metastasis, to remain dormant for a prolonged time, or to undergo degeneration or regression.

From the variety of experiments performed in these studies, results obtained are consistent with the conclusions that (*a*) LMW dextran, as well as dextrans of higher molecular weight, markedly augment the incidence and size of hepatic metastases; (*b*) increasing circulating blood volume enhances the number and size of metastases; and (*c*) contrary to anticipation, *specific* changes in blood viscosity cannot of themselves be related to the incidence of metastases.

While the HMW, and even perhaps the MMW, dextrans may augment metastases as a result of trapping tumor cells in the sluggishly flowing capillary circulation, the mechanism whereby LMW dextran augments hepatic metastases is less evident. This effect seems, at least in part, to occur independently of rheologic changes induced by the dextran. The increased incidence and size of metastases following the inoculation of tumor cells with LMW dextran as a vehicle substantiate this view. No significant alterations of viscosity, hematocrit, or blood volume accompanied this procedure.

Since we have repeatedly demonstrated that liver damage induced by mechanical trauma (12, 22), hepatotoxic agents (12, 19), alterations in hepatic circulation (17), and other modalities (20, 21) augments metastases, consideration was given to the possibility that dextran might have a similar effect. A lack of consistent alteration of the SGP-T seems to discredit this contention. The swelling of the Kupfer cells and sinusoidal compression by retained dextran may have resulted in tumor cell trapping similar to that observed in animals with fatty livers produced by a high fat, choline-free diet or to that following the use of various reticuloendothelial blocking or stimulating agents (e.g., Thorotrast, Proferrin, etc.). Of interest was the observation that LMW dextran is an effective stimulator of the RE system. Its influence on metastases is in this regard analogous to that of other stimulators of the RE system examined by us (20, 21). The lack of alteration of hepatic enzymes noted with dextran suggests that its effect in this regard may be mediated by sinusoidal compression resulting from its deposition in Kupfer cells. The necessity for estimating the actual functional state of the RE system by clearance methods when colloidal or other particles are identified within its cellular components is further emphasized by these findings.

Another suggestion (L.-E. Gelin, personal communication) for the increased tumor incidence following dextran administration is that perhaps this agent *prevents* the formation of microthrombi around endothelial-adherent tumor cells without, in contrast to heparin and fibrinolysin, dislodging them. Consequently, such cells may be better nourished by their more intimate contact with the circulation than they might be if they were surrounded by a clot. Thus, more cells may survive and propagate. Indeed, Smith and Whitfield (43) have demonstrated viable intravascular cells *without* surrounding thrombus formation as long as 7 years after radical mastectomy.

As a result of information accumulated by us, another explanation to account for the mechanism whereby dextran and other fluids that increase blood volume might augment metastasis suggested. Evidence is available to suggest that when tumor cells do attain an extravascular position they may, possibly through motility and flow of interstitial fluid, be transported through organs and tissues to main lymph channels (i.e., the thoracic duct) and, hence, back to the blood vascular system. Thus, it is speculated that the greater the interstitial fluid and lymph flow, the more likely are tumor cells not to become permanently lodged in perivascular spaces. Since the dextrans, and to a lesser extent saline, increase colloidal-osmotic pressure and translocate interstitial fluid, there may be a decrease in lymph flow. Consequently, fewer tumor cells, upon reaching an extravascular position, are dispersed from the organs and, hence, the greater the tumor growth observed. Further studies to assess this concept are in progress.

C. M. Rudenstam (personal communication) suggested that the mechanism(s) whereby mechanical trauma, as repeatedly demonstrated by us (12, 16, 22) and others (1, 4, 38, 39, 42) augments liver metastases might be related to significant alterations in the rheologic properties of blood with an increase in viscosity, similar to the findings of Gelin (23) following tissue injury in the dog. While failure to demonstrate such changes in hematocrit and viscosity by the method of measurement employed following liver damage does not obviate the possibility that at low flow rates in the sinusoids alterations do occur, there are at least no overt findings to substantiate this thesis. If changes in peripheral flow or coagulation mechanisms, or both, resulting from trauma were responsible for the increased metastatic growth, it might have been anticipated that LMW dextrans would inhibit this effect. Our findings following dextran administration militate against this view. Indeed, it has been reported (1) that following trauma slightly more metastases were found in animals treated with LMW dextran than in controls.

While it certainly would be precarious to translate the findings from this experimental model to the situation of humans, it is reasonable to speculate what effect dextran, or other colloid-osmotic agents that increase blood volume, might have when employed during neoplastic surgery—a time when circulating tumor cells increase in number. From these studies, and others alluded to, it might be surmised that employment of the dextrans at such a time is unwise.

Acknowledgment

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Effect of Low Molecular Weight Dextran on Hepatic Metastases in the Rabbit[1]

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SUMMARY

The administration of low molecular weight dextran resulted in an increase in hepatic metastases in rabbits receiving intraportal inoculations of V2 carcinoma cells. This effect is similar to that observed previously in our laboratory following intraportal injection of Walker tumor cells in the rat. Although the mechanism whereby dextran produces such an effect on tumor growth is unclear, the findings do not appear to be related to an anaphylactoid reaction due to dextran. They further emphasize the importance of local ("soil") factors in metastasis formation since the effect observed on hepatic metastases apparently differs from that purported to occur in the lung. The increase in hepatic metastases in two species following dextran administration provokes caution concerning its use in patients undergoing surgery for neoplastic disease.

INTRODUCTION

In the course of obtaining information relative to the effect of a variety of hematorheologic alterations on experimental metastasis formation we observed (3) that the incidence and size of hepatic metastases were increased with administration of low, medium, and high molecular weight dextrans. It was proposed that these findings might be consequent to the increase in circulating blood volume resulting from the dextran infusion. Reasons for such a consideration were presented. Recently, however, Wood *et al.* (10) have challenged this explanation. Since they failed to alter the frequency of pulmonary metastases in the rabbit by dextran treatment, they suggested that our findings obtained in the rat may have been the consequence of a species-specific reaction to dextran which has been described in that animal (1, 7). In spite of our failure to observe such a response in our studies, it was deemed advisable to reassess the effect of low molecular weight dextran on experimental liver metastasis in another species. Consequently, the rabbit has been employed in these investigations. We are unaware of any reports indicating an anyphylactoid response to dextran in this animal.

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MATERIALS AND METHODS

New Zealand female rabbits weighing 1–2 kg housed in individual cages and fed Purina laboratory chow and water *ad libitum* were employed. Tumor cell suspensions from V2 carcinomas propagated in this laboratory for many generations were prepared so that there were 50,000 cells/ml of either normal saline or low molecular weight dextran (Rheomacrodex, Pharmacia Laboratories, Upsala, Sweden, Lot No. 301164), molecular weight 40,000, 10% w/v in normal saline. Animals were randomized into 14 groups with 3 in each, and all groups were treated similarly. One member (A) was injected via a jugular vein with low molecular weight dextran (15 ml/kg body weight), and one hour later under anesthesia (Diabutol) was inoculated intraportally with 1 ml (50,000 cells) of a cell suspension in dextran. A second animal (B) of the group received a jugular vein injection of normal saline (15 ml/kg body weight) followed in an hour by an intraportal tumor cell inoculation (1 ml) prepared in saline. The third rabbit (C) received no jugular vein inoculation prior to the intraportal injection of a similar number of tumor cells in saline. Each of the 3 animals was injected with tumor cells from the same tumor. A different tumor was employed for each of the 14 groups. All groups were sacrificed 8 weeks following injection, and a complete autopsy was done on every animal. Animals dying prior to sacrifice were likewise examined. Tumor growth in livers of each animal in a group was evaluated and compared with that in livers of the other 2 in the *same* group. Size and number of nodules and degree of replacement of liver were taken into consideration. Only when the extent of tumor in one liver was obviously greater than in the other was there judged to be a difference. Lungs were examined for metastases and, when present, were arbitrarily graded as 1+ when they contained a few scattered small nodules, 3+ when all lobes were extensively involved, and 2+ when the amount of tumor was intermediate.

RESULTS

All animals in 11 of the 14 groups survived until sacrifice. In 9 of the 11 sets rabbits receiving dextran demonstrated more liver tumor than did those members which were either injected with saline or were uninoculated prior to intraportal tumor cell injection (Table 1). In one set (No. 8), while the liver of the dextran animal was almost completely replaced by tumor and the saline and uninfused control rabbit had in-

Table 1

Set	Group A, dextran nodules		Group B, saline nodules		Group C, uninfused nodules		Liver tumor comparison	Lung tumors		
	No.	Size (cm)	No.	Size (cm)	No.	Size (cm)		Dextran	Saline	Uninfused control
1	2	2.0, 3.0	0	0	1	1.2	A > C > B	0	0	0
2	5	0.1–1.0	0	0	0	0	A > B = C	0	0	0
3	2	1.5, 2.0	Died	1 day	1	2.0	A > C	0		0
4	6	1.0–5.0	3	1.0–4.5	0	0	A > B > C	1+	1+	0
5	Replaced		1	1.5	1	1.0	A > B > C	1+	0	0
6	1	3.0	14	1.0–2.5	Died	1 day	B > A	0	1+	
7	Replaced		1	1.0	0	0	A > B > C	3+	0	0
8	Replaced		>15	1.0–2.8	>15	0.5–2.7	A = B = C	2+	2+	3+
9	9	0.4–2.5	5	1.0–1.3	1	2.0	A > B > C	0	0	0
10	1	3.3	0	0	0	0	A > B > C	3+	0	0
11	2	0.3, 0.5	0	0	Died	30 day (neg)	A > B	0	0	
12	1	0.5	Almost replaced		5	1.4–4.0	B > C > A	0	3+	1+
13	2	1.0, 1.5	0	0	1	0.8	A > C > B	0	0	0
14	1	1.5	0	0	0	0	A > B = C	1+	0	0

Effect of dextran on experimental hepatic metastasis in the rabbit.

numerable large and small *discrete* nodules involving all lobes, the amount of tumor was so great in all that there was considered to be no difference between them. Only in one set (No. 12) was the amount of tumor in livers of the saline and uninfused controls greater than that found in the livers of the dextran-treated animal. In 2 of the 3 sets in which a non-dextran-treated member died prior to sacrifice, more liver tumor was present in the dextran animal than in its surviving control.

Whereas livers of all 14 animals injected with dextran contained tumor, 6 of 13 receiving saline and 5 of 12 uninfused controls were free of implants. When single or multiple nodules occurred in several members of a set, they were usually more numerous and/or larger in the dextran animals (Fig. 1, Set 4). In several groups the difference was striking (Fig. 2, Set 7).

In 6 of the 11 complete sets it was judged that the livers of saline animals had more tumor than did those in uninfused animals. In 2 sets the opposite was found, and in 3 sets no significant difference was discernible.

Lung metastases were slightly more prominent in the dextran-infused animals. Whereas 43% of those animals demonstrated such tumor, 30% of the saline-injected and 16% of the noninfused controls had metastases. In general, lung tumors were found in those animals having the most extensive hepatic involvement with tumor.

None of the animals in this investigation demonstrated anaphylactic manifestations. Blood pressures were monitored in 6 normal rabbits via femoral artery catheters during and following administration of dextran in the amounts employed in these studies. No significant alteration of blood pressure was observed in any animal. Histologic examination of livers from these animals revealed no abnormality. Sinusoidal congestion was not present.

DISCUSSION

The present findings in the rabbit are coincident with our previous observations in the rat that low molecular weight dextran enhances the incidence and growth of hepatic metastases, and they minimize the possibility that findings in the rat were the result of an anaphylactoid response to the dextran. None of the rabbits in this study exhibited such a reaction.

Other studies reported relative to the influence of dextran on metastasis formation have primarily been concerned with its effect on tumor growth in lung. Results have been variable. While Griffen and Aust (6) noted a decreased incidence of lung metastases in mice following low molecular weight dextran administration, they observed that when metastases did occur they were more numerous and larger than in control animals. Alexander and Altemeier (2) reported that low molecular weight dextran had no beneficial effect in preventing metastases in wounds from hematogenously disseminated tumor cells. In fact, "slightly more metastases were found in animals treated with low molecular weight dextran than in control animals." Schatten *et al.* (9) found that clinical dextran (average molecular weight 75,000) when given prior to V2 tumor cell inoculation markedly reduced the number of lung and liver metastases and increased the survival time of rabbits. In their investigations tumor cells were inoculated via the femoral vein rather than the portal vein which was used in this study. Moreover, their observation that as many as 80% of control animals developed liver metastases following inoculation of tumor cells via a systemic vein has not been our experience in either rabbits or rats so injected. Liver metastases under such circumstances have been a rarity. Wood *et al.* (10), as previously noted, failed to observe an alteration of pulmonary metastases in rabbits treated with dextran, and Garvie and Matheson (5) demonstrated that high molecular weight, as well as low molecular weight dextran, promoted the development of lung metastases in rats following intravenous injection of Walker tumor cells. The latter investigators, as we, failed to note any untoward reaction to dextran in rats, but they conceded the possibility that anaphylaxis may have been present but unrecognized. Microscopic evidence of increased cell sedimentation rates suggested to them that dextran affected the aggregation of tumor cells. They concluded that intravascular agglomeration of these cells with their increased arrest in the lung resulted in the enhancement of metastases. Previous studies by us (4) employing ^{51}Cr-labeled tumor cells

failed to reveal evidence indicating that the augmentation of metastases noted with dextran was due to an increase in number of tumor cells trapped in the liver. This variability is also emphasized by the comprehensive studies of the effect of low, medium, and high molecular weight dextrans on pulmonary metastases of a variety of experimental tumors in rats and mice by Rudenstam (8). These divergent findings may be in part due to differences in routes of administration of tumor cells and dextran, loci of metastasis formation, as well as the species and tumors employed. Other variables, such as effects of anesthesia, positions of the rabbit, and different lots of dextran employed may likewise play a role.

It is of interest that a slightly greater number of metastases was observed in this study in rabbits infused with saline than in uninfused controls. We had previously observed (3) a relationship between tumor growth and an increase in circulating blood volume resulting from the administration of low molecular weight dextran, plasma, or saline.

These studies further emphasize the importance of local ("soil") factors in considering metastatic mechanisms. They also emphasize that the findings in one model system utilized to obtain information relative to metastases in one organ (lung) are not necessarily applicable to that of another (liver). Despite the mechanism involved, the finding of increased hepatic metastases following dextran administration in two species suggests that employment of these agents during or after surgery for neoplastic disease may be unwise.

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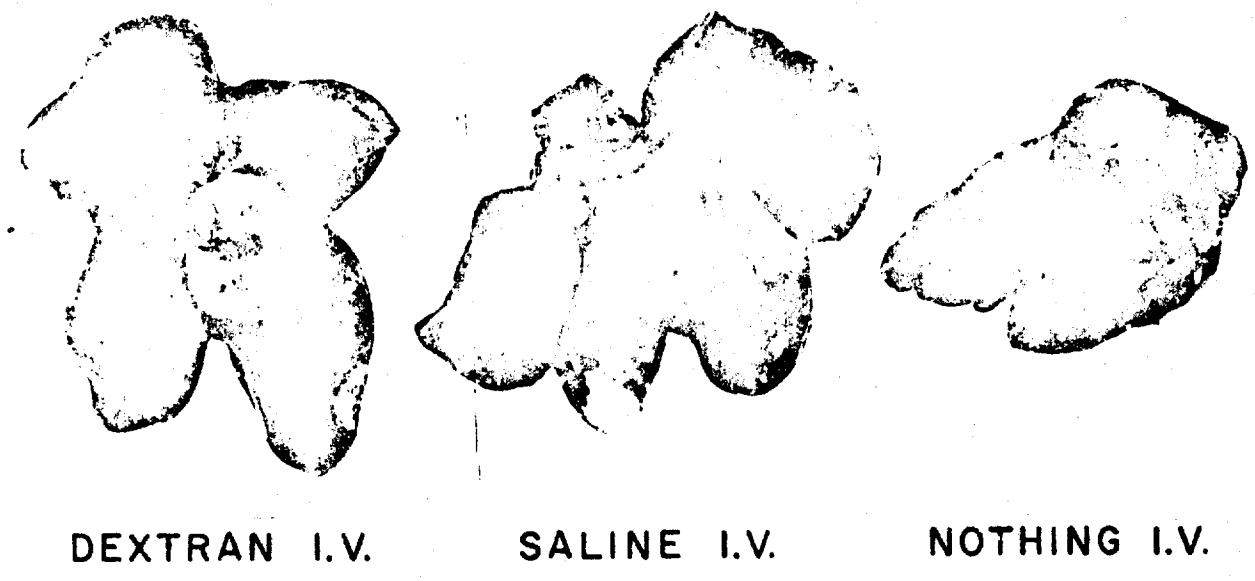

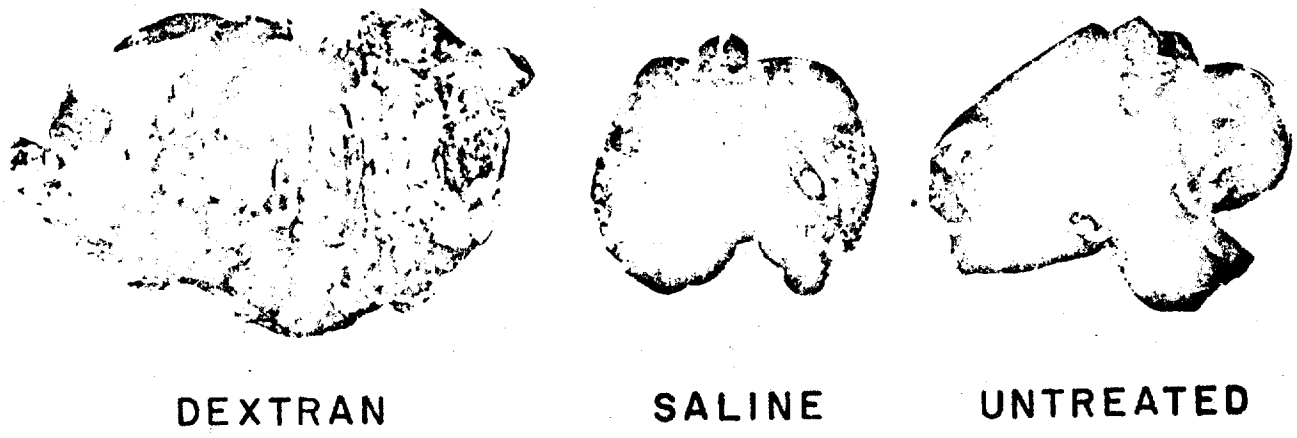

Fig. 1, Set 4. Effect of dextran on hepatic metastasis in the rabbit.
Fig. 2, Set 7. Effect of dextran on hepatic metastasis in the rabbit.

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LOW MOLECULAR WEIGHT DEXTRAN AND EXPERIMENTAL METASTASIS GROWTH

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The effect of low molecular weight-dextran (LMD; M 4×10⁴) was tested on intravenously-induced metastases in a syngeneic tumour-host system in mice. When given as intravenous pretreatment LMD increased the total number of gross extrapulmonary metastases, without significantly changing the planimetrically estimated lung and liver metastases. When, on the other hand, the same volume of LMD was given in the cell suspension, there was a further increase in gross extrapulmonary metastases and also in pulmonary metastases.

There was an interesting parallelism between these results and the effect of cell and systemic treatment with LMD on the subcutaneous transplantability of tumour cells. Both types of treatment increased the frequency of subcutaneous takes but cell pretreatment with LMD alone increased resulting tumour volumes.

The effects of LMD were not reflected in any alteration of the viability index in the exclusion test or in the aggregability of the tumour cells.

The attachment of tumour cells onto the vascular endothelium is an indispensable step in the formation of blood-borne metastases. On the assumption that low molecular weight dextran (LMD) might hinder this attachment by its flow-promoting and anticoagulant properties, the effect of LMD has previously been tested on intravenously induced metastases in some experimental systems. In rats LMD increased the number of gross metastases from Walker 256 carcinoma cells (Fisher and Fisher, 1966; Garvin and Mathison, 1966). Promoting as well as inhibiting effects of LMD on experimental metastases were found with syngeneic rat tumours, while LMD reduced the number of pulmonary takes from a mouse tumour (Rudenstam, 1967). In rabbits given V2 carcinoma cells intravenously LMD either did not alter metastasis formation (Wood *et al.*, 1967) or increased it (Fisher and Fisher, 1968).

The evaluation of these conflicting results is hampered, apart from differences in experimental design, by the consistent use of tumours giving rise to metastases only in the lungs and/or liver. The absence of manifest metastases in other organs may give a false picture of changes induced in the tumour cell distribution (Hagmar and Boeryd, 1969). The use of rats, in which dextran may provoke an "anaphylactoid reaction" (Kato and Gözsy, 1960), and in most cases allogeneic tumour-host systems, also renders the interpretation of results difficult.

The aim of the present investigation was to test the effect of LMD on intravenously-induced metastases in a syngeneic tumour-host system, where metastases can be recorded in other organs besides lungs and liver. The study includes a comparison between LMD pretreatment of animals and LMD preincubation of tumour cells. This experimental design serves to test if LMD

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can alter metastasis formation by changing the tumour-cell surface. Special attention was paid to any effect of LMD on the tumour-cell viability. This was checked with dye exclusion and subcutaneous transplantation of a critically small cell dose (Hagmar and Norrby, 1970). A possible action of LMD *via* tumour cell aggregation (Garvie and Matheson, 1966) was also considered.

MATERIAL AND METHODS

The experiments were performed with sarcoma MCG1-SS (Mellgren *et al.*, 1966) in syngeneic CBA mice. The tumour cell suspensions were prepared under sterile conditions by trypsin and DNase (Boeryd *et al.*, 1965). To obtain monodisperse suspensions and retain maximal viability, the suspensions were spun down and resuspended in Parker 199 medium supplemented with 10% syngeneic mouse serum ($Parker_{90}$-$serum_{10}$) plus 0.6 mg per ml DNase (Norrby *et al.*, 1966). To the same end the suspensions were always stored at 0° C (Knutson *et al.*, 1971). The cell counts were performed in a haemacytometer. At least forty fields (1/160 mm^3) were counted from each sample.

Dye exclusion and aggregability tests

Cell suspensions were diluted with $Parker_{90}$-$serum_{10}$ to about 10^6 cells per ml. After accurately assessing the cell number by renewed counting, 1 ml of the suspension was transferred to a 10 ml sterile, unsiliconized glass tube containing 1 ml of 10% LMD in saline (Rheomakrodex, Pharmacia, Uppsala, Sweden); mean molecular weight 4×10^4. One ml of the same cell suspension was added to 1 ml saline as a control. The sealed tubes were stored on ice and shaken gently by being turned upside down five times every 15 min. Samples for counting were taken immediately after shaking. After 1 and 2 h the Trypan blue dye exclusion test was performed on samples from the two types of suspensions. The viability index was calculated as the number of unstained cells/the total number of cells. After the 2 h count the suspensions were fixed by adding 4% buffered formaldehyde to a final concentration of 0.4%. Such fixation preserves the degree of aggregation (Norrby *et al.*, 1966). From the fixed suspensions a final cell count was made with attention directed towards the frequency of aggregates. When calculating the cell number the dilution induced with formalin was corrected for. Differences in cell number were calculated in percentages.

Subcutaneous transplantability

To test the transplantability of MCG1-SS cells after exposure to 5% LMD three experiments were performed:

Experiment 1: Suspensions with and without LMD in Parker $_{95}$-$serum_5$ containing 10^3 cells/ml were stored on ice for 2 h, and gently shaken as described above. One tenth ml of each suspension was injected subcutaneously into both groins of 12 adult CBA mice. To obtain an exact dosage a 100µl syringe was used.

Experiments 2 and 3: These experiments were identically designed but performed consecutively. Suspensions with or without 5% LMD, containing 10^3 cells/ml, were stored on ice for 2 h as above. From each suspensions 0.1 ml was injected bilterally into the groins of six mice. Six mice served as additional controls, being injected bilaterally with the LMD-free suspension after having received 0.05 ml 10% LMD in saline subcutaneously in the neck.

The animals were examined daily for the development of palpable tumours. The tumour takes in each groin of the same animal were registered separately. The observation period was 21 days in experiments 1 and 2, 19 days in experiment 3. At these times the animals were killed and resulting tumours weighed. The incidences of tumours were compared with chi-square analysis and tumour weights with Student's t-test.

Metastasis study

The experimental plan is shown in Table I. Suspensions in Parker $_{95}$-$serum_5$ with and without 5% LMD were injected into a tail vein. Each animal received 0.1 ml of suspensions containing 5×10^5 cells/ml immediately after the 0.1 ml of LMD or saline given as intravenous pretreatment.

The period of observation was 15 days. The mice were sectioned and gross metastases noted. The lungs and livers were prepared for histological examination as described by Boeryd (1965). The number of metastases per cm^3 tissue, their mean volume in mm^3, and their total volume in per cent of organ tissue were planimetrically estimated (Boeryd *et al.*, 1966).

Wilcoxon's two-sample rank test was used to compare differences between groups in the

TABLE I

DESIGN OF METASTASIS STUDY

Group	No. of animals	Subcutaneous pretreatment	Suspension medium (with percentages indicated)
Controls	10 (♀ + ♂)	0.1 ml saline	Parker 199-saline $(1:1)_{ss}$-serum$_s$
LMD pretreatment	10 (♀ + ♂)	0.1 ml saline/LMD (1:1)	Parker 199-saline $(1:1)_{ss}$-serum$_s$
LMD in suspension	10 (♀ + ♂)	0.1 ml saline	Parker 199-LMD $(1:1)_{ss}$-serum$_s$

number and volumes of lung and liver metastases. Incidences of metastases were compared by chi-square analysis. The mean numbers of gross extrapulmonary metastases were compared as described previously (Hagmar and Boeryd, 1969). Differences in all experiments with $p<0.05$ were accepted as significant.

RESULTS

Cell excretion and aggregability tests

There was no decline in cell viability in LMD-containing or control suspensions according to the trypan blue test during the 2 h storage of the suspensions at 0° C. There was usually an insignificant fall in cell number, similar in the two types of suspensions. Results from one of the four experiments performed are shown in Table II. The suspensions contained mainly single cells, i.e. no aggregates of cells were observed in the counting chamber.

Cell transplantability

Experiment 1: As seen from Figure 2, the tumours developed earlier from the LMD-containing than from the control suspension. The final incidence of tumours also became greater ($p<0.05$) and resulting tumours larger ($p<0.05$) from the LMD-exposed cells.

Experiments 2 and 3: The tumours developed somewhat earlier in experiment 3, causing spontaneous death of one control mouse and two mice receiving LMD-containing cell suspension. This necessitated the termination of this experiment on day 19. Differences obtained were very similar in the two experiments, however, permitting the demonstration of the combined results in Figure 2.

The tumour development began first from LMD-exposed cells. Animals receiving the same dose of LMD subcutaneously apart from the cells soon caught up, however, and the final tumour incidence became similar in these two groups, greater than in the controls ($p<0.05$). The tumour weights became greater when LMD was given with the cells than when it was given separately ($p<0.005$). In this respect the controls were intermediate and significantly different from the two LMD-groups ($p<0.05$).

Metastasis study

The incidence, number and location of gross extrapulmonary metastases are shown in Table III, while pulmonary and hepatic metastases are accounted for in Table IV.

LMD pretreatment of animals (Group II) increased the mean number but not the total incidence of gross extrapulmonary metastases. In

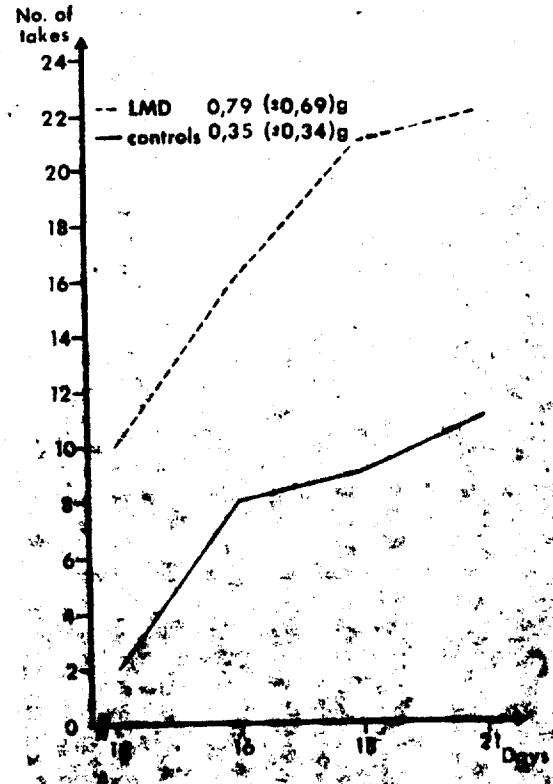
TABLE II

CELL NUMBER AND VIABILITY INDEX DURING STORAGE OF MOGI-SS CELL SUSPENSIONS AT 0°C
FOR 2 h. VIABILITY INDEX = NUMBER OF UNSTAINED CELLS/TOTAL NUMBER OF CELLS

Suspension	Original cell number per ml ×10⁶ and viability index in parentheses	Viability index 1 h	Cell number per ml and viability index in parentheses 2 h
Parker-saline $(1:1)_{ss}$-serum$_s$	4.00 (0.92)	0.88	3.72 (0.89)
Parker-LMD $(1:1)_{ss}$-serum$_s$	4.00		

lungs and liver there were only minor, numerical deviations from the controls, without statistical significance.

LMD, when present in the cell suspension (Group III), increased the total incidence of gross extrapulmonary tumour takes compared to the controls. The mean number of extrapulmonary metastases was greater than in the controls ($p<0.001$) and than in Group II ($p<0.01$). In the lungs there was a significant increase in the total volume of metastases and their number tended to be larger ($0.15<p<0.10$) than in the controls. Liver metastases showed no statistical deviations from controls with $p<0.20$.



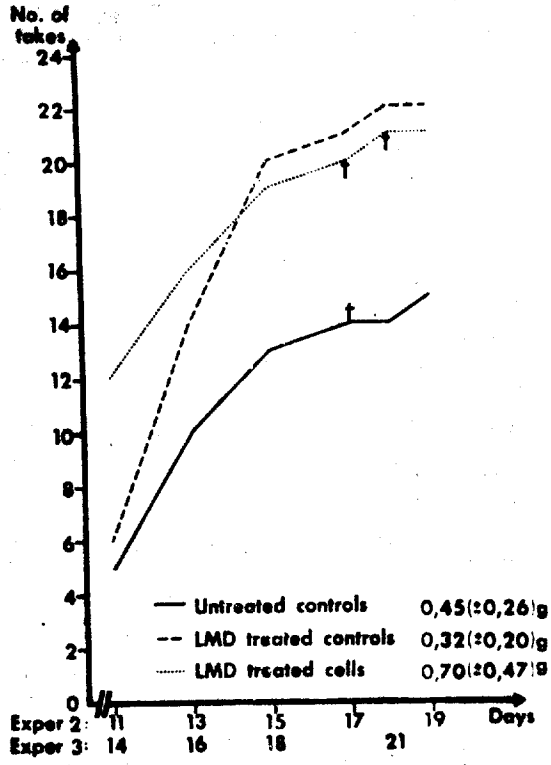


FIGURE 2

Number of tumour takes and tumour weights (±SD) after transplantation subcutaneously of 100 MCG1-SS cells in 0.1 ml medium. The cell suspensions in $Parker_{90}$-$serum_{10}$ diluted 1:1 with 10% LMD in saline (LMD-treated cells) or saline were stored on ice for 2 h prior to injection. LMD-treated controls received 0.05 ml 10% LMD in saline subcutaneously in the neck, apart from the tumour cells. Combined results of Experiments 2 and 3 with 12 mice in each group injected bilaterally. Crosses indicate spontaneous deaths in Experiment 3.

DISCUSSION

In the present study the pretreatment of animals with LMD did not alter the amount of metastases in the lungs or liver, but increased the number of tumour takes in other organs. This type of change is of course only detectable in systems where the tumours give rise to metastases in several organs. The increase of the total metastasis crop may be compared, however, with the increase of lung or liver metastases obtained with larger dextran doses

TABLE III

GROSS METASTASES TO OTHER ORGANS THAN LUNGS AND LIVER AFTER IV INJECTION OF $5 \cdot 10^4$ MCG1-SS CELLS. IF MORE THAN ONE METASTASIS PER ORGAN IN AN ANIMAL, THE TOTAL NUMBER OF TAKES IS GIVEN IN PARENTHESES

	Group I Controls	Group II LMD (pretreatment)	Group III LMD (in suspension)
No. of mice with metastases	6/10	9/10	10/10 [2]
Mean No. of metastases per mouse	2.1	4.0 [1]	6.5 [1]
Incidence and No. of metastases in:			
Subcutis	3	6 (12)	6 (11)
Mediastinum	1	3	3 (5)
Mesentery	2	2 (5)	5 (7)
Pelvic fat	6 (8)	3	7 (10)
Retroperitoneal fat	1	1	3 (4)
Skeletal muscle	1	4 (7)	3 (8)
Ovaries	2	2	3
Lymph nodes	3	1	3 (6)
Kidneys	0	3	5
Adrenals	0	1	1
Salivary glands	0	1	1
Uterine corpus	0	1	1 (4)

[1] Significant differences from controls ($p < 0.01$).
[2] Significant differences from controls ($p < 0.05$).
Levels of significance are not given for the various organs.

TABLE IV

METASTASES TO LUNGS AND LIVER AFTER IV INJECTION OF 5×10^4 MCG1-SS CELLS

Group	To lungs				To liver			
	Incidence	Mean volume, mm^3	Mean number per cm^3	Percentage tumour in organ	Incidence	Mean volume, mm^3	Mean number per cm^3	Percentage tumour in organ
I Controls	7/10	0.26	59	1.5	5/10	1.1	14	1.6
II LMD (pretreatment)	8/10	0.38	55	2.1	5/10	1.2	13	1.5
III LMD (in suspension)	8/10	0.23	135	3.1 [1]	8/10	1.2	18	2.2

[1] Significant difference from controls ($p < 0.05$).

in rats, with or without preceding trauma (Gelin and Rudenstam, 1966; Fisher and Fisher, 1966; Garvie and Matheson, 1966; Rudenstam, 1967).

Results in rats are hard to evaluate, however, because of the "anaphylactoid reaction" towards dextran, which may occur in that species. This reaction involves an increased capillary permeability with peripheral oedema and haemoconcentration which *per se* may influence the fate of the injected tumour cells (Fisher and Fisher, 1966, 1968; Rudenstam, 1968). In spite of this, Fisher and Fisher (1966, 1968) consider the plasma volume expansion due to the colloid osmotic pressure of dextran as a major factor among the systemic effects which lead to an increment of metastases. Gelin and Rudenstam (1966) suggested that LMD might improve the nutrition of tumour cells caught in white thrombi by increasing the capillary blood flow. Among other systemic effects of possible importance, an

alteration of the intimal surface by dextran (Bloom *et al.*, 1964) and an interaction with the RES (Fisher and Fisher, 1966) have been discussed.

In rabbits, which are free from the "anaphylactoid reaction", conflicting results have been obtained with the V2 carcinoma, perhaps because of variations in the experimental design. Wood *et al.* (1967) were unable to influence V2 metastases with MMD (mean molecular weight dextran, $M = 7 \times 10^4$) or LMD but performed the treatment exclusively *after* the tumour-cell injection. Schatten *et al.* (1965) claimed a reduction of V2 metastases in MMD-treated rabbits. The effect was more pronounced with treatment before than after the tumour cell injection. Mixing the tumour cells with MMD before injection lessened the reducing effect on metastases. Fisher and Fisher (1968) obtained more V2 metastases in animals pretreated with LMD when the tumour cells were at the same time suspended in LMD.

Thus, there are some indications from studies in rabbits that dextran may augment metastasis formation by affecting the tumour cells directly. The present study in mice offers further evidence in this direction, since there was an increase of the total volume of pulmonary metastases from LMD-exposed cells compared to controls. In addition, there was a further increase of gross extrapulmonary metastases in comparison to the increment obtained with the same dose of LMD given as intravenous pretreatment.

The results of course may be interpreted in terms of a promoted lodgement of tumour cells in vessels. Thus LMD might, in systemic treatment and still more by pretreatment of cells, promote the sticking of tumour cells at sites where they are able to grow into metastases. Analogies with other cell types such as erythrocytes and thrombocytes, to which LMD seems to confer a higher negative surface charge (Seaman *et al.*, 1965) and reduced adhesiveness (Bygdeman *et al.*, 1966; Richter, 1966) make such an interpretation rather unlikely, however.

Gastic and Matheson (1966) suggested that increased tumour-cell trapping would result from intravascular tumour-cell aggregation by LMD. This explanation remains entirely hypothetical, however, even in their own system. For a concentration was used for intravenous metastasis induction, and the aggregation took place in a serum-free medium. In the present study there was no indication whatsoever that LMD aggregated MCG1-SS cells in a serum-containing medium.

The present study instead offers some evidence that LMD may increase metastasis formation by an altered transplantability of the tumour cells. For there is a striking parallelism between the ways in which LMD affected tumour growth from intravenously and subcutaneously administered tumour cells.

When MCG1-SS was transplanted subcutaneously in a critically small cell dose, LMD pretreatment of cells increased not only the number of tumour "takes", but also the volumes of resulting tumours. A similar increment in frequency of takes was obtained by giving the same dose of LMD as systemic treatment. This type of treatment, however, if anything reduced the weights of resulting tumours. These findings seem to indicate that systemic effects of LMD, apart from direct effects on the tumour cells, may play a role in the survival of the cells in the animal host. A direct cell effect must also be operative, however, promoting the growth of tumours.

The nature of this hitherto undescribed effect of LMD in a syngeneic system remains as yet a matter of speculation. It may offer a clue, however, to the seemingly paradoxical increase of metastases found in several systems, although it contrasts with a decreased transplantability of V2 carcinoma in rabbits (Armstrong and Cohn, 1966) and an impaired cell growth *in vitro* in dextran-containing medium (Powell, 1961). Nor was it reflected in the viability index by dye exclusion in the present study. This is admittedly a very crude method of assessing cell viability. The discrepancy between the two ways of testing tumour-cell vitality might, however, reflect that it is in relation to the host and not unspecifically that LMD favours the survival of the tumour cells. Defense mechanisms of the host such as phagocytosis might for instance be affected. Immunological mechanisms are likely to play a minor role in the survival of transplants from a serially transplanted tumour such as MCG1-SS in syngeneic hosts. It cannot be excluded however that LMD, by changing the tumour-cell surface, may interact in some phase of an, albeit weak, immunological defence reaction. The results

[illegible] in this respect, be compared with those [illegible] (1968) with [illegible] or alteration of surface [illegible] [illegible].

[illegible] will be the subject of further studies to define [illegible] by what means LMD and other dextrans [illegible] tumour growth and metastatic spread. [illegible] mechanisms involved may be of general [illegible] for the survival of disseminated tumour cells and also have some implications for the clinical situation in humans.

ACKNOWLEDGEMENTS

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DEXTRANE A FAIBLE POIDS MOLÉCULAIRE ET CROISSANCE MÉTASTATIQUE EXPÉRIMENTALE

L'effet du dextrane à faible poids moléculaire (LMD; $M\,4\times10^4$) a été étudié sur des métastases induites par voie intraveineuse dans un complexe hôte-tumeur syngénique murin. Administré en prétraitement intraveineux, le LMD a fait augmenter le nombre total de métastases extrapulmonaires macroscopiques sans modifier de façon significative le nombre de métastases hépatiques et pulmonaires estimé par planimétrie. Par contre, lorsque le même volume de LMD a été ajouté à la suspension cellulaire, on a constaté un accroissement plus fort des métastases extrapulmonaires macroscopiques et, en outre, une augmentation des métastases pulmonaires.

Un parallèle intéressant a été tiré entre ces résultats et l'effet du traitement au LMD des cellules et de l'organisme sur la transplantabilité sous-cutanée des cellules tumorales. Les deux types de traitement ont accru la fréquence des prises sous-cutanées, mais seul le prétraitement des cellules au LMD a fait augmenter le volume des tumeurs [illegible].

Les effets du LMD ne se sont pas traduits par une quelconque altération de l'indice de viabilité dans les tests de coloration ni par une aptitude particulière des cellules tumorales à [illegible].

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A.M.A. ARCHIVES OF PATHOLOGY

Carcinogenic Studies on Water-Soluble and Insoluble Macromolecules

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Numerous experimental investigations of the past decade have established the fact that various synthetic and semisynthetic, water-insoluble carbon and silicon polymers elicit sarcomas in rats and mice at the site of their subcutaneous or retroperitoneal implantation (Table 1).[1-23] Experimental studies of more recent years have shown that this property is shared by two water-soluble carbon polymers, namely, polyvinylpyrrolidone (PVP) and carboxymethylcelluiose, when parenterally introduced into rats. Sarcomas developed at the site of their repeated subcutaneous injection in an aqueous solution, while reticulum-cell sarcomas of the liver and peritoneal lymph nodes represented the main neoplastic responses to the subcutaneous and intraperitoneal deposition of PVP in powder form or after its repeated intravenous introduction in saline solution into rats and mice.[24-26]

The target organs of carcinogenesis from water-soluble carbon polymers are under these conditions the tissues in which the macromolecular material is retained and stored and in which according to previous observations with these and other water-soluble synthetic, semisynthetic, and natural carbon polymers (polyvinyl alcohol, methylcellulose, pectin) a marked proliferation of reticuloendothelial cells and histiocytes accompanies the striking storage phenomena as well as the arterial atheromatosis developing after the parenteral administration of these colloids.[27-32] These proliferative and thesaurismotic reactions involving in part also the parenchymal cells of the liver, kidney, and brain and elicited by nonphysiologic macromolecules bear a close resemblance to those present in a number of spontaneous thesaurismoses which are characterized by the retention of lipoids, polysaccharides, mucopolysaccharides, and proteins of normal and abnormal types and of which some are associated with the occurrence of benign or malignant neoplastic lesions. Such physiologic thesaurismoses with neoplastic characteristics or complications are the various lipoidoses, such as essential xanthomatosis; Gaucher's disease; Niemann-Pick disease; the myocardial glycogenosis often accompanied by tumors of the brain, skin, and kidney; the mucopolysaccharidosis known as gargoylism; the microproteinosis associated with myelomatosis, and the megaloglobulinosis of Waldenström frequently associated with cancers of various organs but particularly those of the reticuloendothelial system. The experimental "polymer cancers" developing around the various synthetic and semisynthetic carbon and silicon polymers exhibit moreover a definite relationship to the pulmonary cancers induced in human lungs by the retention and in the vicinity of inhaled asbestos dust, which is a silicon oxide polymer.

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TABLE 1.—*Carcinogenic Macromolecular Carbon and Silicon Polymers*

Name	Description	Investigators
	I. Water-Insoluble Polymers	
	(a) Hydrocarbon Polymers Synthetic	
Polythene	Polyethylene	Oppenheimer et al., Druckrey et al., Bering & Handler
Lucite	Polymethylmethacrylate	Oppenheimer et al., Laskin et al., Zollinger
Polystyrol	Polyvinylbenzol	Oppenheimer et al.
Ivalon	Cross linked polyvinyl alcohol	Oppenheimer et al.
Dacron	Polyester condensate of terephthalate & ethylene glycol	Oppenheimer et al.
Bakelite	Phenol-formaldehyde condensate	Turner
	(b) Halogenated Hydrocarbon Polymers (Synthetic)	
Saran	Polyvinylene chloride	Oppenheimer et al.
PVC, Igelit, Vestolit, Vinnol	Polyvinyl chloride	Oppenheimer et al.
Teflon	Polyfluor (chlor)-olefine	Oppenheimer et al.
Pliofilm	Polymethylmethacrylate chloride	Oppenheimer et al.
Vinyon N, Dynel	Copolymer of vinyl chloride and acrylonitrile	Oppenheimer et al.
	(c) Aminised Hydrocarbon Polymers (Polyamides)	
Nylon	Polyhexamethylene diamine adipanide	Oppenheimer et al.
Perlon	Poly-e-caprolactam, polyurethane	Druckrey et al.
	(d) Hydrocarbon Polymers (Semisynthetic & Natural)	
Rubber	Processed latex gum	Druckrey et al., Mecke
Cellophane	Processed polyglucose (cellulose)	Oppenheimer et al., Druckrey et al., Körbler & Frank
Linen		
Parchment paper	Processed cellulose	Oppenheimer et al.
Silk		Oppenheimer et al.
Keratin		Oppenheimer et al.
Ivory		Nothdurft
	(e) Silicon Polymers	
Silastic	Polydimethylsiloxane processed (silicon rubber)	Oppenheimer et al., Mohr & Nothdurft
	II. Water-Soluble Polymers	
PVP	Polyvinylpyrrolidone	Hueper Lusky & Nelson
CMC, Collocel	Carboxymethylcellulose	Lusky & Nelson
Tween 60	Polyoxyethylene sorbitan monostearate	Lusky & Nelson

The discovery of polymer or macromolecular carcinogenesis has created important and urgent scientific as well as practical problems. A major controversy has developed concerning the type and the specificity of the causative mechanism active in the development of these polymer cancers. Some investigators (Druckery, Hueper) favored the view that specific chemical or physicochemical properties of these macromolecular agents are responsible for and active in the carcinogenic process. Such qualities may be related to specific end-groups, to macromolecular peroxide cross linkages, to metabolic fragments or molecular chemical abnormalities, to residual valencies, to trapped free radicals, to complex formation with protein or mucopolysaccharides or with their building stones or to their molecular size and shape, or to the presence of impurities, such as traces of the catalyst used in their production (Haddow, Fitzhugh). Other workers (Nothdurft, Oettel, Oppenheimer et al.) have concluded that the great chemical diversity of the polymers involved in carcinogenesis and the apparent dependence of this property upon the absolute size of the implants and upon the size of the unbroken surface of the implants of water-insoluble polymers and on their physical status—i. e., film, disk, fabric, thread or powder—militates against a specific chemical causal mechanism of polymer carcinogenesis. They have advanced the concept that the carcinogenesis of the polymerized substances depends upon their ability to elicit in the tissues the formation of a fibrous capsule from which in turn as the result of chronic nonspecific irritation and therefrom resulting continued cell proliferation sarcomas originate on a multicentric basis. The tendency toward the formation of a fibrohyaline capsule depends chiefly, according to these investigators, upon the physical status of the polymer, being most marked when polymers are implanted as films and being least developed when the polymer is present in powder form. Polymer carcinogenesis, according to this concept, is a nonspecific physical phenomenon, which is, in the opinion of its supporters (Nothdurft, Oppenheimer et al.), essentially analogous to the sarcogenesis obtained around disks of certain metals follow-

TABLE 2.—*Carcinogenic Metals and Mineral Foils and Powders*

Name	Description	Investigators
Mica	Metal silicates	Oppenheimer et al.
Glass ?	Metal silicate	Oppenheimer et al.
Quarts	Silicon oxide polymer	Druckrey et al.
Asbestos	Metal silicon oxide polymer	Druckrey et al.
Mercury		Druckrey et al.
Silver		Nothdurft
Gold		Nothdurft
Platinum		Nothdurft
Stainless steel	Alloy of nickel, chromium, iron	Oppenheimer et al.
Steel		Oppenheimer et al.
Tantalum		Oppenheimer et al.
Vitallium	Alloy of cobalt & chromium	Oppenheimer et al.
Beryllium		Barnes et al., Gardner; Dutra et al.
Nickel		Hueper
Chromium		Hueper, Schinz
Cobalt		Heath, Schinz
Uranium		Hueper

ing their implantation into the subcutaneous tissue of rats (Table 2).[33-39]

Apart from the distinct scientific importance which these two divergent concepts on polymer carcinogenesis possess, they are also of immediate practical significance, since they must influence the carcinogenic screening techniques applied to polymers, as well as the assessment of possible carcinogenic hazards connected with exposure to the same polymer when present in different physical states and when incorporated in or in contact with consumer goods or when introduced into experimental animals or man by various routes.

The second and practical problem of major importance resulting from these observations on polymer carcinogenesis is closely linked to their use in the human economy. Water-soluble and insoluble macromolecular polymers of various types are being introduced into the human environment to a rapidly increasing degree and are being employed in numerous consumer goods, such as medicinal and cosmetic preparations, medicinal protheses, food and medicinal containers, packagings and coatings, household and sanitary goods, paints, dinnerware, and clothing. The human population, therefore, has frequent and intimate contact with polymers. The observations made on experimental animals, therefore, raises the question whether or not carbon and silicon polymers coming in contact with human tissues by various routes and in different forms may have a carcinogenic effect also upon man. Because of the possibility of a potential carcinogenic hazard to man posed by previous studies on polymers, a greatly extended experimental study of carbon and silicon polymers for elucidating the chemical and physical properties underlying their carcinogenicity and their carcinogenic mechanism was urgently needed.

Experimental Procedure

The experiments to be reported were conducted for these reasons on various water-soluble and insoluble carbon and silicon polymers. Ten PVP's were tested. Of these, four were of technical grade and three were of medicinal type, while three others were specially prepared for this study by American and German manufacturers. These three PVP's had a rather low average molecular weight, ranging from 10,000 to 23,000. Two of them, moreover, had a narrow molecular weight range. The average molecular weights of the entire PVP series ranged from approximately 10,000 to about 300,000. Six of them were made in Germany; three, in the United States, and one, in Great Britain. Different catalysts were used in their production, depending upon the manufacturer.

In an additional experiment, a polyvinyl alcohol preparation of American make and with an average molecular weight of 120,000 was used.

A third series of experiments comprised the testing of various polyglucoses. Eleven different dextrans manufactured by an American pharmaceutical concern and by the Regional Laboratory in Peoria, Ill., were used. These dextrans not only varied in molecular weight which covered a range between 37,000 and several million, but they differed among each other also in regard to their molecular shape. Some consisted of linear molecules, while others showed varying degrees of branching and thus had an almost globular shape. Other polyglucoses tested were a brand of methylcellulose with an average molecular weight of 140,000, an ethylcellulose of rather low molecular weight, and a soluble starch.

In addition to ethylcellouse the water-insoluble polymers studied were as follows: 1. Silastic, which is a processed polymer of dimethlysiloxane. This is vulcanized by the addition of 2,4-

TABLE 3.—*Carcinogenic Tests on Macromolecular Substances—Water-Soluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Species	Animals, No.
PVP 1	Technical	20,000	Subcut.	200	200	C57bl. mouse	30
PVP 2		20,000	Powder				
PVP 3		50,000	Intraper. powder	200	200	C57bl. mouse	30
PVP 4		300,000	Subcut. powder	500	500	Beth. bl. rat	20
			Intraper. powder	500	500	Beth. bl. rat	20
			Intraven. 7% sol.	18.5	150	Beth. bl. rat	15
			Intraven. 7% sol.	2,800	23,000	Dutch rabbit	3-6
PVP 6	Medicinal	50,000	Intraper.	1,000	15,000	Beth. bl. rat	20
PVP 7		50,000	20% sol.				20
			Intraven.	3,500	56,000	Dutch rabbit	6
			7% sol.	3,500	56,000	Dutch rabbit	6
			Subcut. powder	200	200	Beth. bl. rat	20
			Subcut. Rep. powder	200	600	Beth. bl. rat	20
Plasmosan		50,000	3.5% sol.	1,700	12,600	Dutch rabbit	6
PVP 5	Special	10,000	Subcut.	200	260	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
			Intraper.	200	200	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
PVP K17		18,000	Intraper.	500	2,000	Beth. bl. rat	20
PVP K25		23,000	25% sol.	6,250	62,500	Dutch rabbit	6
Polyvinyl alcohol	Technical	120,000	Subcut. powder	500	500	Beth. bl. rat	25
Methylcellulose	Technical	140,000	Subcut. powder	500	500	Beth. bl. rat	25
Soluble starch	Technical		Subcut. 45% sol.	500	503	Wistar rat	30
			Intraper.	500	500	Wistar rat	30
Dextran 1	Linear	200,000	Subcut.	200	700	C57bl. mouse	40
Dextran 2	Linear	100,000	Powder	500	500	Beth. bl. rat	20
Dextran 8	Branched	37,500	Intraper.	200	200	C57bl. mouse	20
Dextran 9	Branched	300,000	Powder	500	500	Beth. bl. rat	20
Dextran 10	Branched	80,400	Intraven.	1,750	17,500 to 35,500	Dutch rabbit	3-4
Dextran 11	Highly br.	71,400	7% sol.				
Dextran 3		Sev. million	Subcut.	200	200	C57bl. mouse	35
Dextran 4	Linear	75,000	Subcut.	200	200	C57bl. mouse	30
Dextran 5	Highly br.		Subcut.	200	200	C57bl. mouse	28
Dextran 6	Highly br.		Subcut.	200	200	C57bl. mouse	13
Dextran 7	Highly br.		Subcut; powder	200	200	C57bl. mouse	9

dichlorbenzoyl peroxide. Through the action of this chemical on the latex the dimethylsiloxane polymer molecule is altered from a straight linear molecule into a net molecule because of the formation of numerous cross linkages between the macromolecular chain molecules. The average molecular weight of the thereby resulting silicone rubber ranges from 300,000 to 400,000. The rubber contains, moreover, as a filler and plasticizer, a specially prepared and very pure silicon dioxide, which in the silicon rubber takes the place of carbon black used in the manufacture of natural or synthetic carbon rubber. 2. Polyethylene. 3. Polyurethane (Mondur TD-80), which is a modified polymer of tolylene-diisocyanate.

Pertinent data relative to the various macromolecular substances used as well as information on the general scheme of experimental investigations applied are presented in Tables 3 and 4.

The various water-soluble macromolecules were investigated for carcinogenic properties by administering them in single or repeated doses either as powders or as aqueous solutions through subcutaneous, intraperitoneal, and intravenous routes so as to determine the possible role which differences in chemical composition as well as in molecular size and shape might play in bringing about these responses. The water-insoluble polymers, on the other hand, were studied for ascertaining whether differences in the physical state of these substances —i. e., whether their presence in the form of cubes, balls, sheets (disks), films, sponges, or powders— is important in determining the occurrence and degree of cancerous reactions to parenterally implanted water-insoluble polymers. Rabbits, rats, and mice were employed for obtaining information on the species-specific character and range of such manifestations.

TABLE 4.—*Carcinogenic Tests on Macromolecular Substances—Water-Insoluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Physical State	Species	Animals, No.
Ethylcellulose	Technical	Low viscosity	Subcut.	500	500	Powder	Beth. bl. rat	25
Polyethylene			Intraper.	65	65	Cube 3.5×3.5×3 mm.	Beth. bl. rat	30
				65	65	Sheet 0.5 mm. thick, disk 12 mm. diam.	Beth. bl. rat	30
				65	65	Film 10×17.5 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polyurethane			Subcut.	65	65	Sheet 2mm. thick 2 disks 3mm., 5mm. diam.	Beth. bl. rat	30
				65	65	Sponge 25×20×3 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
			Intraper.	65	65	Sheet	Beth. bl. rat	30
				65	65	Sponge	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polydimethylsiloxane (latex) (gum)		High viscosity	Subcut.	300	300	Ball	Beth. bl. rat	30
			Intraper.	300	300	Ball, 5 mm. diam.	Beth. bl. rat	30
Polydimethylsiloxane rubber (Silastic)			Subcut.	300	300	Cube	Beth. bl. rat	30
			Intraper.	300	300	Cube 5 mm. diam.	Beth. bl. rat	30
Silica			Subcut.	300	300	Powder	Beth. bl. rat	30
			Intraper.	300	300	Powder	Beth. bl. rat	30

The aqueous solutions of the polymers used were prepared with isotonic saline and were autoclaved for insuring their sterility. PVP (Plasmosan), as well as the solutions of PVP K17 and PVP K25, was supplied in ampules. The maximal observation period for mice and rats was two years; for rabbits, up to four years. All survivors were killed at the end of this period. Autopsies were performed on all animals. Histologic examinations of the tissues were made on all animals exhibiting grossly demonstrable pathologic changes or on at least 30% of animals of each series regardless of the presence or absence of such lesions. The organs of all rabbits were studied histologically. Normal untreated animals of the same strain and animals given parenteral implants or injections of various noncarcinogenic vehicles (wool fat, gelatin, tricaprylin) or test chemicals served as controls. They were permitted to live for the same length of time as the animals of the experimental series and were used for determining the type, age, and site distribution of spontaneous tumors.

The neoplastic lesions observed among the animals of the different test series in relation to the species and polymers used and the routes of their administration employed are listed in Table 5.

Experimental Observations

In the assessment of the relative significance of the cancers observed in the various experimental series in relation to the different polymers administered, the following criteria were considered:

(*a*) The absolute and relative number of cancers in a specific test series.

(*b*) The number of animals in a particular test series.

(*c*) The degree of uniformity of neoplastic response in several test series in which the same polymer was given to the same species by different routes.

(*d*) The degree of uniformity of neoplastic responses in test series in which different species were employed and to which the same polymer was administered.

(*e*) The topographical distribution of cancers in specific organs and tissues and their histogenetic types in their relation to the sites of retention and/or storage of the polymer introduced, i. e., the identity of the site of the implanted or stored polymer with the site of the subsequently developing proliferative hyperplastic and precancerous lesions and cancers.

(*f*) The relative frequency of spontaneously occurring cancers of the same type and site in control series of normal untreat-

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

Chemical Molecular Weight	Route Admin.	Species	No. Animals	Sites & Types: Lymph Nodes: Lymphoma	Lymph Nodes: Leukemia	Liver: Ret. Sarcoma	Liver: Cholangioma	Liver: Ret. Sarcoma	Liver: Leukemia	Lung: Adenoma	Lung: Mesothelioma	Heart: Mesothelioma	Breast: Adenofibroma
PVP 1	Subcut.	Mouse	50										
	In. per.		50										
30,000	Subcut.	Rat	30			4		2					
	In. per.		30			4	1						
	In. ven.		15			1		1					
	In. ven.	Rabbit	6										
PVP 2	Subcut.	Mouse	50	3									
	In. per.		50					1					
20,000	Subcut.	Rat	30				2						
	In. per.		30			1							1
	In. ven.		15										1
	In. ven.	Rabbit	4										
PVP 3	Subcut.	Mouse	50										
	In. per.		50	3									
80,000	Subcut.	Rat	30			5		2	1			1	
	In. per.		30				1		1			1	
	In. ven.		15	3									
	In. ven.	Rabbit	3										
PVP 4	Subcut.	Mouse	50	1									
	In. per.		50										
300,000	Subcut	Rat	30			4		2				1	
	In per.		30			6							1
	In. ven.		15			2							
	In. ven.	Rabbit	2										
PVP 5	Subcut.	Mouse	30										
10,000	In. per.		30	1								1	
	Subcut.	Rat	20			1		3					
	In. per.		20			2		1					
PVP 6	In. per.	Rat	20	Unfinished									
	Subcut.	Rat	30			4		3					
80,000	Rep. sbc.		30			1		1					
	In. ven.	Rabbit	6										
PVP 7	In. per.	Rat	30	Unfinished									
80,000	Subcut.	Rat	30					2					
	Rep. sbc.		30			2		1					2
	In. ven.	Rabbit	6										
Plasmosan 80,000	In. ven.	Rabbit	6										
PVP K17	In. per.	Rat	35	Unfinished									
18,000	In. per.	Rabbit	6										
PVP K25	In. per.	Rat	35	Unfinished									
23,000	In. per.	Rabbit	6										
PVA 120,000	Subcut.	Rat	25				1	4			1		2
Methocel 140,000	Subcut.	Rat	25										
Dextran 1	Subcut.	Mouse	40	1	1			2					
200,000	In. per.		20	1									
	Subcut.	Rat	20			2		1					2
	In. per.		20			1							2
	In. ven.	Rabbit	3										
Dextran 2	Subcut.	Mouse	40					1					
100,000	In. per.		20					4		2			
	Subcut.	Rat	20			1						1	1
	In. per.		20				1	2					
	In. ven.	Rabbit	3										
Dextran 3	Subcut.	Mouse	35	1						1			
Sev. million													
Dextran 4 75,000	Subcut.	Mouse	30			1							

of Tumors

Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors					
								Total		Benign		Malig.	
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%
								0					
								0					
						1		7	23	1	3	6	20
	1		2	2				10	33	3	10	7	23
			1					6	20			6	20
								0					
								3	6			3	6
								1	2			1	2
			1	1				4	13	3	10	1	3
			1	3				6	20	5	17	1	3
			1					2	13	2	13	1	1
								0					
								0					
				1		1		3	6			3	6
			3	1				11	37	2	7	9	30
			2				1	7	28	2	7	5	16
								6	40	1	7	5	33
								0					
								1	2			1	2
								0					
	1		1	2				11	37	2	7	9	30
			2					9	30	1	3	8	27
			1					3	20			3	20
								0					
								0					
								1	3			1	3
			2					6	20			6	20
								4	20			4	20
			2					7	23			7	23
			6					8	27			8	27
								0					
								2	7			2	7
			2					7	23	2	7	5	16
								0					
			1					9	36	3	12	6	24
			1					1	4			1	4
								4	10			4	10
								1	5			1	5
			2					7	25	2	10	7	35
			1					4	20	2	10	2	10
								0					
								1	2			1	2
								6	30	2	10	4	20
								3	15	1	5	2	10
			1					4	20	1	5	3	15
								0					
								2	6	1	3	1	3
								1	3			1	3

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

Chemical Molecular Weight	Route Admin.	Species	No. Animals	Sites & Types: Lymph Nodes: Lymphoma	Lymph Nodes: Leukemia	Liver: Ret. Sarcoma	Liver: Cholangioma	Liver: Ret. Sarcoma	Liver: Leukemia	Lung: Adenoma	Lung: Mesothelioma	Heart: Mesothelioma	Breast: Adenofibroma
Dextran 5 H. Br.	Subcut.	Mouse	28			3							
Dextran 6 H. Br.	Subcut.	Mouse	13										
Dextran 7 H. Br.	Subcut.	Mouse	9			1							
Dextran 8	Subcut.	Mouse	40	1									
37,000	In. per.		20	1									
	Subcut.	Rat	20				1		1				1
	In. ven.	Rabbit	3										
Dextran 9	Subcut.	Mouse	40			1							
300,000	In. per.		20	1	1								
	Subcut.	Rat	20										
	In. per.		20			2		2					
	In. ven.	Rabbit	3										
Dextran 10	Subcut.	Mouse	40	1				2					
89,400	In. per.		20					4					
	Subcut.	Rat	20			1	1	3					1
	In. per.		20				1	2					
	In. ven.	Rabbit	3	1					1				
Dextran 11	Subcut.	Mouse	40					2	1				
71,400	In. per.		20										
	Subcut.	Rat	20					1					
	In. per.		20			2							1
	In. ven.	Rabbit	3							2			
Starch	Subcut.	Rat	30										6
	In. per.		30			1							1
Eth. cel.	Subcut.	Rat	25							1			
Silastic	Subcut.	Rat	30				2	1			2		1
rubber	Int. per.		30										
Polysil. latex	Subcut.	Rat	30										1
	Int. per.		30			2					2		
Silica	Subcut.	Rat	45			1	1	2			2		
	Int. per.		30					2					1
Polyurethane	Subcut.; int. per. disk; sponge; powder		6×30	Unfinished									
Polyethylene	Int. per. cube; disk; film 4×30; powder			Unfinished									
Normal controls		Rat	200			7		4			1		3
Treated controls		Mouse	840			6							

ed animals or those treated with noncarcinogenic chemicals.

When evaluating the data contained in Table 4 by applying the above listed criteria, carcinogenic responses attributable to the action of the polymer parenterally introduced into mice, rats, and rabbits are present for the following polymers: PVP 1, PVP 3, PVP 4, PVP 5, PVP 6, Dextran 10, Silastic rubber, and PVA. Such a relationship is probable but not definite for PVP 2, PVP 7, Dextran 1, Dextran 2, Dextran 5, Dextran 11, Polysilicone latex, and silica. For all other polymers, although some of them belong to the same general chemical group as the ones adjudged to be carcinogenic, the

a Parenteral Introduction of Water-Soluble and Insoluble Polymers—Continued

of Tumors															
Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors							
								Total		Benign		Malig.			
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%		
								3	10			3	10		
								0							
								1	10			1	10		
								1	2			1	2		
								1	4			1	4		
			1					4	20	2	10	2	10		
								0							
								1	2			1	2		
								2	10			2	10		
			2	1				3	15	1	5	2	10		
			1					5	25			5	25		
								0							
								3	8			3	8		
								4	20			4	20		
1			1					8	40	2	10	6	30		
			1					4	20	1	7	3	13		
								2	70	1	35	1	35		
								3	7			3	7		
								0							
			1					2	10			2	10		
			1					4	20	1	7	3	13		
								2	66	2	66				
								6	20	6	20				
					1		1	4	12	2	6	2	6		
			2					8	12			8	12		
		10						16	55	3	10	13	43		
								1	3			1	3		
		1						2	6	1	3	1	3		
			1					5	17			5	17		
			1	1				8	18	2	4	6	14		
			3					6	20	1	3	5	17		
1			4		1			20	10	3	1	17	9		
								6	0.7			6	0.7		

experimental observations failed to demonstrate carcinogenic properties.

Whenever the cancer rate in a test series was found to be two to three times that of the control series, and especially when such a ratio was present following the introduction of a particular polymer by several routes and into several species and whenever the cancers involved organs and tissues in which the particular polymer had elicited storage phenomena and hyperplastic and precancerous cellular responses related to these reactions, the macromolecular substance in question was considered as possessing carcinogenic properties. The evidence

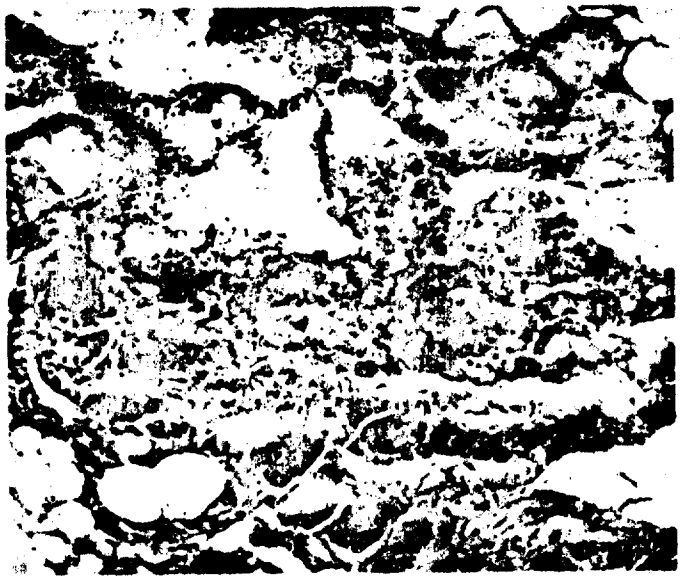

Fig. 1.—Fat tissue with giant cells filled with clumps of blue-stained matter (PVP) (rat).

underlying these conclusions is presented in the data listed in Table 5.

The histopathologic findings pertinent in this respect for the different polymers investigated are as follows:

I. Polyvinylpyrrolidones.—(*a*) Thesaurismotic Reactions: Storage reactions which were characterized by the appearance of bluish-stained material representing probably a PVP protein or mucopolysaccharide complex in usually swollen cells were observed in many organs and tissues of mice, rats, and rabbits, regardless of the molecular weight of the various PVP's tested. The degree of storage reactions depended mainly on the relative amounts of PVP administered and varied considerably between different series of animals and between animals belonging to the same series. In general they were most marked in rabbits, particularly those intravenously injected with Plasmosan and PVP's 6 and 7, i. e., the medicinal PVP's tested. They were somewhat less pronounced in rats and affected least the mice.

Fat tissue at the site of implantation contained often blue-stained phagocytic cells scattered throughout an otherwise normal tissue. Occasionally foci of huge multinu-

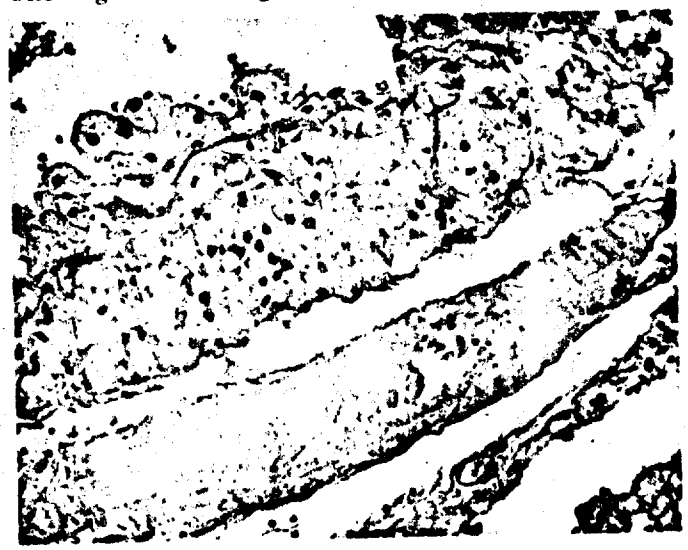

Fig. 2.—Meningeal membrane with foam cells with blue tinted cytoplasm (PVP) (rabbit).

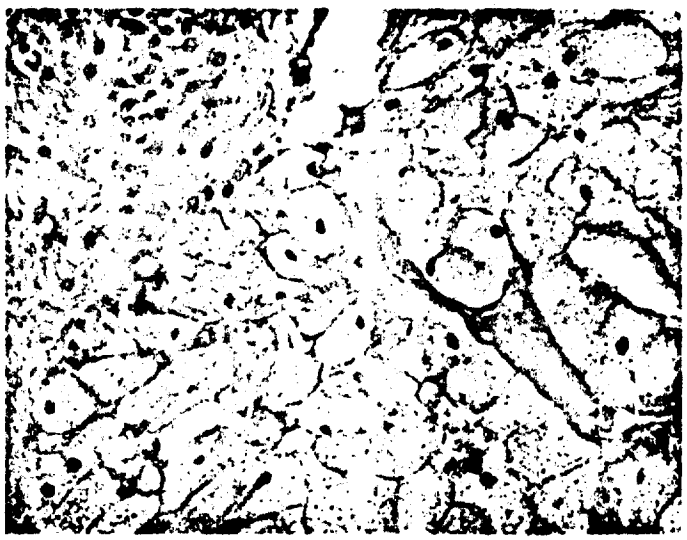

Fig. 3. — Choroid plexus with massive proliferation of foam cells with bluish stained cytoplasm; glia cells with blue cytoplasm in adjacent brain tissue (PVP) (rabbit).

cleated foam cells with bluish cytoplasm were found in the fat tissue (Fig. 1).

Massive accumulations of blue matter containing foam cells were noted in the meninges of rabbits given PVP 6 and 7 and Plasmosan (Fig. 2). In some animals this condition was associated with a distinct increase of histiocytic elements in the meninges. The ependymal cells as well as the cells of the choroid stroma were usually balloon-like, had a foamy and often blue-stained cytoplasm, and formed grape-like proliferations in the ventricles (Fig. 3). Groups of multinucleated giant cells were sometimes present in these masses (Fig. 4), while swollen and blue matter containing glia cells were noted at times in the adjacent brain tissue. In a few rabbits and mice also glia and ganglion cells of the basal ganglionic region exhibited a distinct blue stipling of their swollen cytoplasm. Cells with a bluish-stained cytoplasm were occasionally found also within peripheral nerves.

The lungs not infrequently revealed clusters of blue-stained foam cells located in the interstitial tissue. Giant-cell granulomas often were present in pulmonary capillaries occluding their lumens. The lumens of

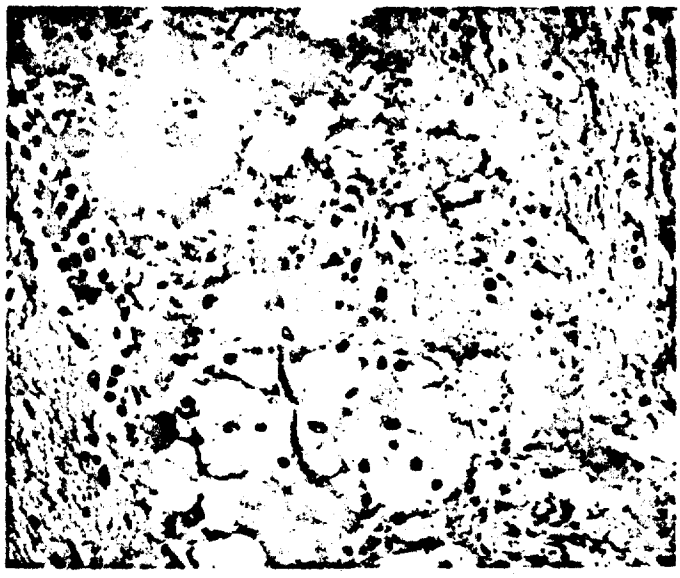

Fig. 4. — Choroid plexus with foam cells and giant cells with smudge-like clumps of blue-stained matter (PVP) (rabbit).

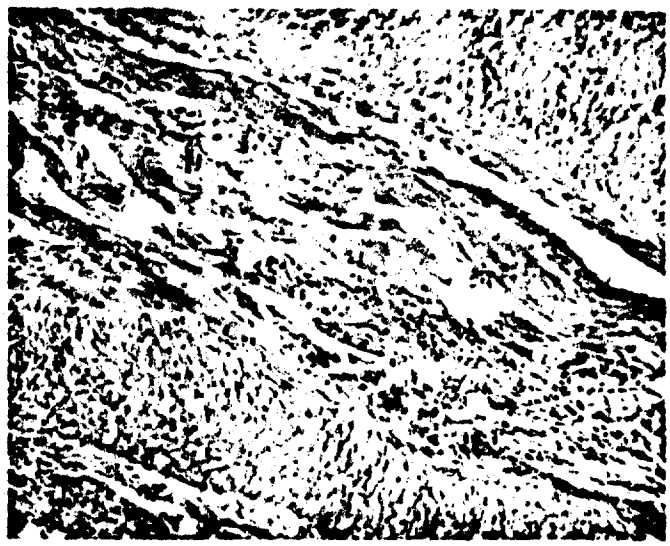

Fig. 5.—Pulmonary artery with an occluding foreign-body giant-cell granuloma (PVP) (rabbit).

medium-sized and large pulmonary arteries sometimes contained similar and often large giant-cell granulomas (Fig. 5).

Retention of PVP in the endothelial cells of the aortic intima of rabbits was not uncommon, producing a blue, bead-like cellular lining and being associated with an increase of endothelial cells. Such reactions sometimes resulted in the production of intimal foam-cell cushions in the ascending aorta, representing an experimental type of atherosclerosis (Fig. 6). Similar reactions were observed in the aortic leaflets as well as in the subendocardial tissue. Myocardial infiltrations with blue-stained foam cells were frequent and were usually most pronounced in the subepicardial area, where they gave rise to multiple focal accumulations of blue-stained foam cells.

Blue-stained matter was often noted in both liver cells and swollen Kupffer cells, which not infrequently were transformed into giant cells. Histiocytic balloon-like swollen blue foam cells were present in the periportal tissue, which occasionally was markedly increased in amount and which contained giant-cell granulomas (Fig. 7).

Mediastinal as well as abdominal lymph nodes revealed at times foci of blue-stained foam cells. Similar alterations occurred in

Fig. 6.—Ascending aorta with an intimal foam-cell cushion containing blue-stained matter (PVP) (rabbit).

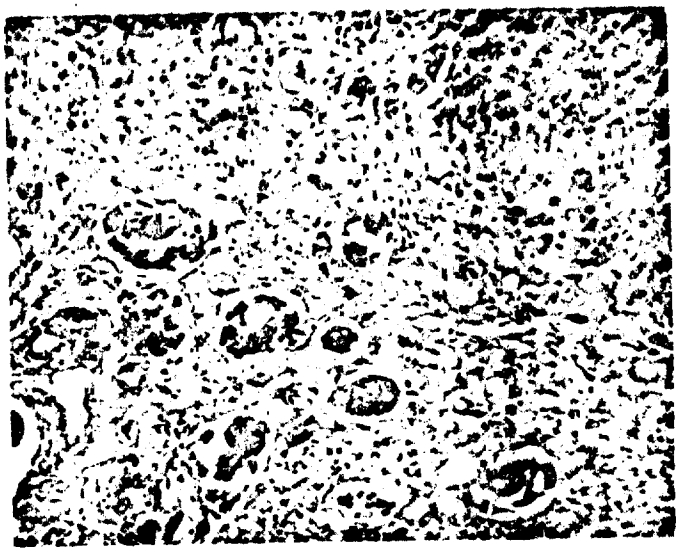

Fig. 7.—Liver of rat with interstitial fibrosis and clusters of foam cells and giant cells containing blue-colored material (PVP).

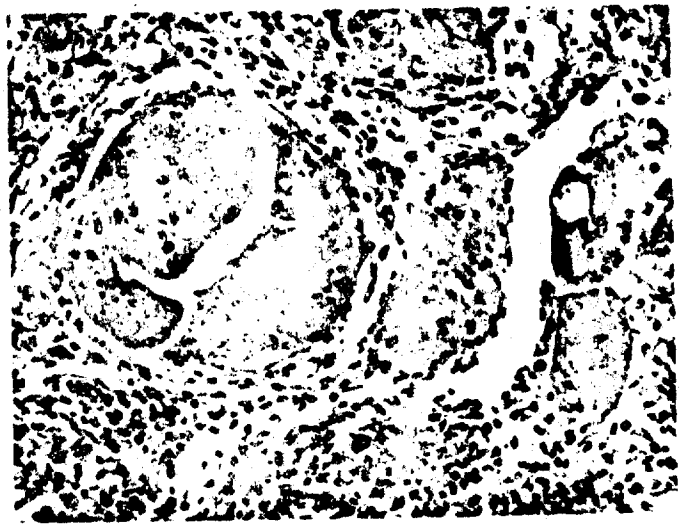

Fig. 8. — Clusters of multinucleated giant cells in the splenic pulp containing blue-stained foam cells (PVP) (rabbit).

Fig. 9.—Kidney of rat with glomeruli composed of foam cells faintly bluish-stained (PVP).

Fig. 10.—Endometrium of rat with interstitial foam cells and giant cells which are deeply blue-stained (PVP).

the spleen, where the pulp contained occasionally masses of huge multinucleated syncytia (Fig. 8), displaying a bluish-stained cytoplasm. "Lakes" of bluish matter apparently originating from a disintegration of reticulum cells were often observed in the bone marrow.

Blue-stained foam cells originating from capillary endothelium were noted in the renal glomeruli (Fig. 9). Bluish matter was contained also in tubular epithelial cells as well as tubular lumens. The tubules affected sometimes showed a hyperchromatic cell lining of syncytial character.

Massive deposits of PVP were often found in the endometrium, where they were located in clusters of foam cells as well as where they were infiltrating the intercellular spaces, thereby coming in close contact with the glandular epithelium (Fig. 10). Blue-stained histiocytes occurred frequently in the ovarian stroma.

It is evident from the evidence present that given adequate amounts of PVP this substance will be distributed diffusely in the body and will be retained in many organs and tissue not only in phagocytic cells but also in the parenchymal cells of several organs, such as the brain, liver, and kidney.

(*b*) Hyperplastic and Precancerous Reactions: Many of the organs and tissues in which PVP was stored displayed hyperplastic reactions which sometimes assumed

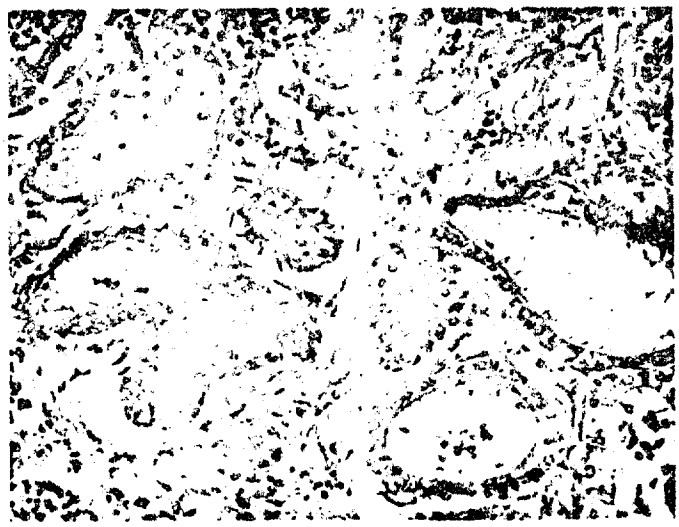

Fig. 11.—Alveolar adenomatosis of the lung in a rat (PVP intraperitoneally).

Fig. 12.—Bronchiolar cyst lined by a stratified squamous-cell epithelium with peribronchiolar adenomatosis in a rat (PVP intraperitoneally).

metaplastic as well as "preneoplastic" characteristics if standards of human cancerology are applied. The storage of PVP in ependymal, choroid, and meningeal cells was always accompanied by marked proliferations of the cells affected.

Multifocal adenomatosis of pulmonary alveoli, particularly in the peribronchial areas, was rather frequently encountered in rats (Fig. 11). In several rats these lesions were associated with the occurrence of multiple cysts lined by a stratified squamous epithelium and originating apparently from bronchial structures (Fig. 12). The pleura as well as the epicardium and endocardium of rats exhibited focal proliferations of closely packed hyperchromatic oval cells, which displayed a tendency to invade the underlying tissue and therefore became suspected of possessing potentially malignant properties or of representing actually early cancerous manifestations.

Similar diagnostic difficulties were met in assessing the potential and actual biologic character of the focal as well as diffuse proliferations of Kupffer cells in liver. These cells possessed sometimes a foamy cytoplasm, at other times they represented round or oval cells arranged often in ill-defined nodules or trabeculae (Figs. 13 and 14). Decisions concerning the benign or malignant nature of some of these reactions were by necessity sometimes of a rather arbitrary nature. The spleen and lymph

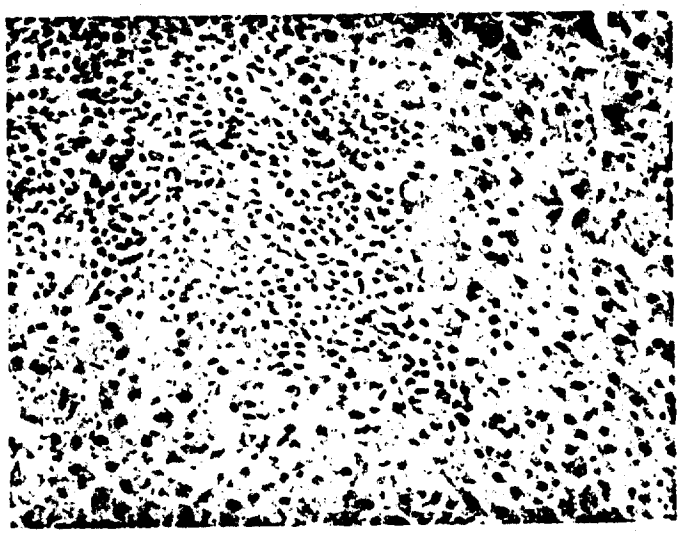

Fig. 13.—Focal Kupffer-cell proliferation in the liver of a rat (PVP subcutaneously).

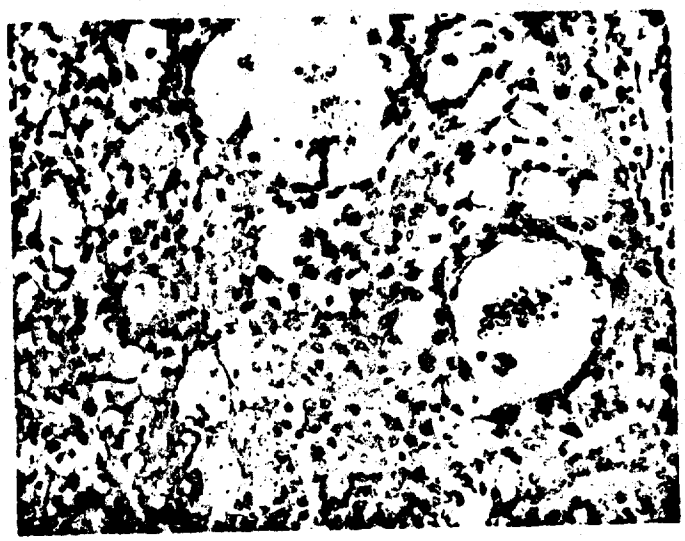

Fig. 14.—Focal Kupffer-cell proliferation of foam cell type in a rat (PVP).

nodes showed in some animals marked proliferations of reticulum cells and an occasional loss of follicular structure.

A marked glandular hyperplasia of the endometrial glands frequently assuming the character of a massive adenomatosis and associated with the formation of cysts, epithelial papillomatosis, and cellular atypia was noted in a considerable number of rats and rabbits. Such glandular proliferations were sometimes coexisting with focal or diffuse squamous-cell metaplasia of the endometrial epithelial lining as well as at times with areas of definite adenocarcinoma or squamous-cell carcinoma (Fig. 15). An unusual degree of cellularity of the endometrial stroma was also observed in some rats with PVP deposits.

Noteworthy in this connection is, moreover, the coexistence of large masses of blue-stained foam cells invading the adjacent muscular tissue located near an osteoid and possibly sarcomatous tumor involving the lower jaw in a rabbit (Fig. 16).

(*c*) Cancerous Reactions: The malignant nature of the lesions adjudged to be cancerous was attested not only by their histologic characteristics but also by their locally invasive growth, their diffuse involvement of the target organ, and often by the frequent presence of widespread metastases.

Fig. 15.—Adenocarcinoma of the uterus in a rat (PVP).

Fig. 16.—Sarcomatoid osteogenic tumor of the lower jaw with groups of phagocytic cells containing blue-stained material (PVP) (rabbit).

17. Endocardial mesothelioma in a rat (PVP).

Fig. 18. — Marked mesothelial proliferation with foam cells of the pleura of probably sarcomatous nature in a rat (PVP).

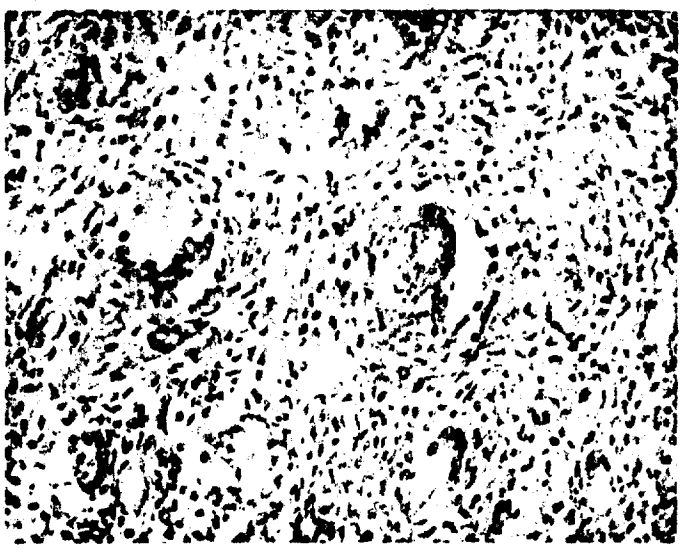

Fig. 19. — Kupffer-cell sarcoma with multinucleated giant cells of the liver in a rat (PVP).

The endothelio- or mesotheliosarcomatous neoplasms originating from the endocardial, epicardial, and pleural lining cells consisted of closely and irregularly packed oval cells invading the myocardium or lung, respectively. They extended along the pulmonary or cardiac vessels into the mediastinum and mediastinal lymph nodes and formed there large white medullary masses (Figs. 17 and 18).

The Kupffer-cell sarcomas displayed usually a similar cellular structure, being composed of strands or masses of oval-shaped cells or of irregularly round cells with a reticulum network. Huge multinucleated giant cells were present occasionally in these cell masses (Fig. 19). In addition to these sarcomatous hepatic lesions a few livers displayed changes of a leukemic character of monocytic or lymphocytic type.

Similar round cellular malignant neoplasms forming nodular masses in the peritoneal lymph nodes and invading the abdominal and thoracic organs apparently originating from the reticulum cells of lymph nodes and only occasionally from those of the spleen. The presence of giant cells in some of these tumors produced a resemblance with histologic changes found

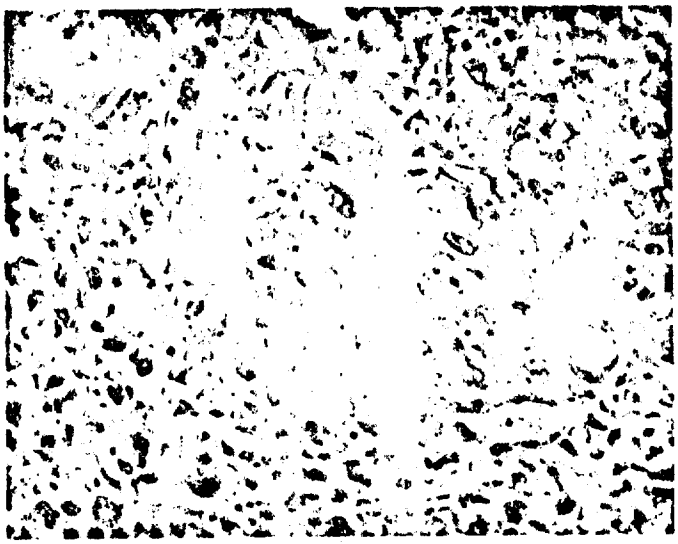

Fig. 20.—Squamous-cell carcinoma and round-cell sarcoma of the uterus of a rat (collision tumor) (PVP).

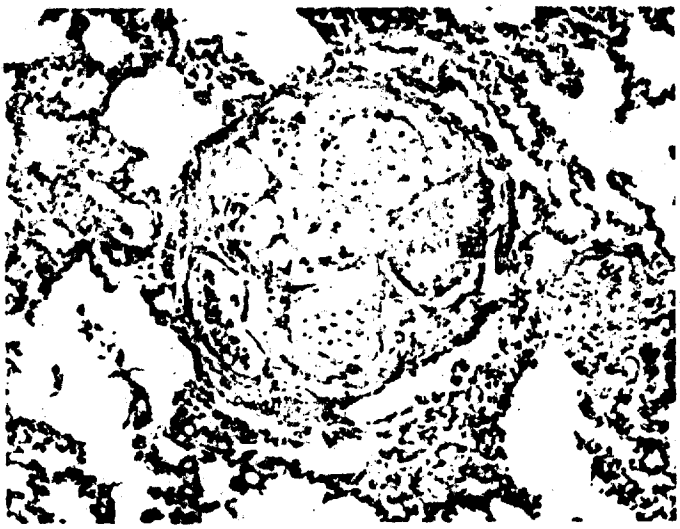

Fig. 21. — Intracapillary cluster of foreign-body giant cells in the lung of a rabbit (dextran).

in sarcomas derived from Hodgkin's disease in man.

The cancers affecting the uterus of some rats and of one rabbit were either adenocarcinomas or squamous-cell carcinomas or anaplastic carcinomas. Occasionally a mixture of adenocarcinoma and squamous-cell carcinoma or of adenocarcinoma and endometrial round-cell sarcoma was observed (Fig. 20).

Two rats developed squamous-cell carcinomas of the skin in the region of the subcutaneous implantation of PVP.

The histopathologic changes recorded for mice, rats, and rabbits which received PVP's by various parenteral routes demonstrate the topographical and in part also cellular identity of the localization of thesaurismotic, hyperplastic, and cancerous reactions. Statistical as well as histopathologic evidence thus support the view that at least some of the cancers observed in these animals are attributable to the PVP administered.

II. Polyvinyl Alcohol.—The storage phenomena observed were essentially identical in type and distribution with those seen in the PVP animals as well as with those previously reported for dogs and rabbits,[38,39] i. e., polyvinyl alcohol was present in vari-

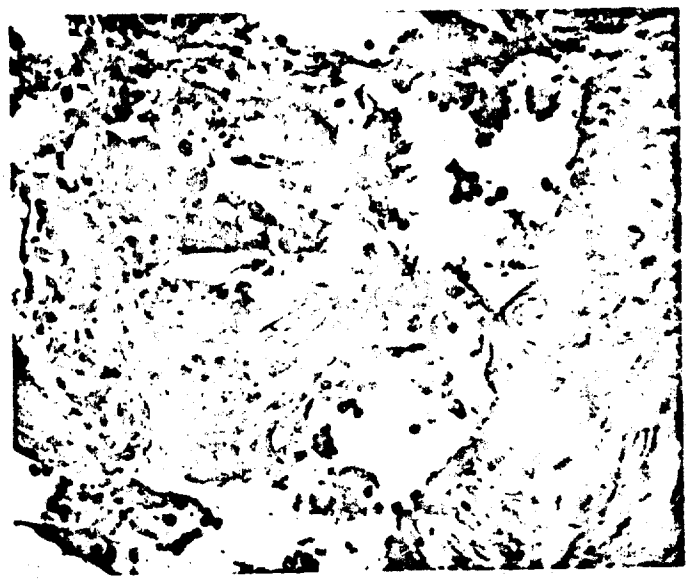

Fig. 22.—Foreign-body giant-cell granuloma obstructing the lumen of a large pulmonary artery in a rabbit (dextran).

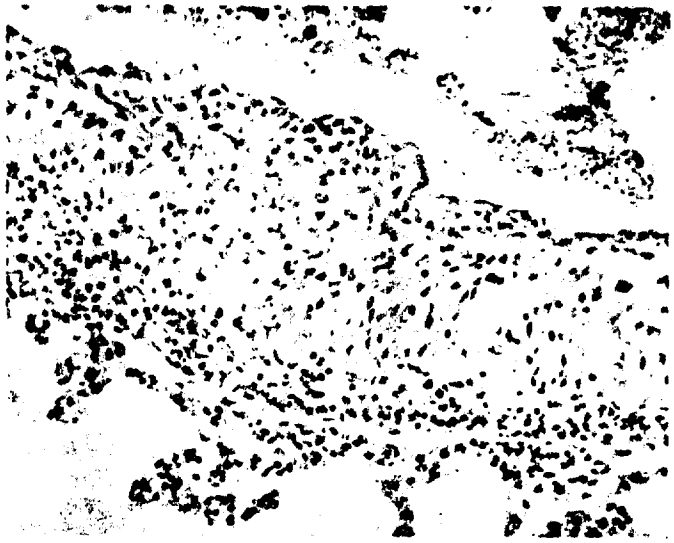

Fig. 23.—Intimal cushion of proliferated endothelial cells in a pulmonary artery of a rabbit (dextran).

ous cellular constituents, such as periportal foam-cell granulomas, isolated and swollen Kupffer cells, proliferated reticulum cells and giant cells of the spleen, and interstitial giant-cell granulomas of the lung. The cancerous reactions likewise resembled the corresponding ones observed among the PVP animals, i. e., Kupffer-cell sarcomas and mesotheliomas.

III. Dextran.—(*a*) Thesaurismotic Reactions: The lungs of rabbits revealed frequently intracapillary giant-cell granulomas, which were sometimes rather cellular and at other times mainly hyaline (Fig. 21). Similar but much larger formations occasionally blocked the lumens of large pulmonary arteries (Fig. 22). In the presence as well as in the absence of such foreign-body granulomas the endothelial lining of arteries exhibited sometimes focal increases of cells which at times produced small crescent-shaped, cellular plaques of the intima (Fig. 23). Apparently older lesions of this derivation appeared as fibrous intimal thickenings (Fig. 24).

Foam-cell accumulations in intrahepatic sinusoids were only occasionally observed in the livers of mice (Fig. 25). In the spleen of one rabbit hyaline masses surrounded atrophic lymph follicles. The same rabbit exhibited also an extensive hyalinosis of the renal glomeruli.

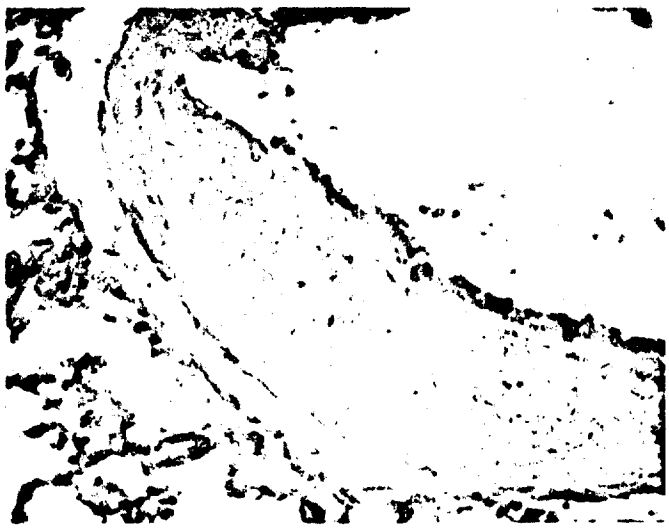

Fig. 24.—Fibrous intimal thickening in a large pulmonary artery of a rabbit (dextran).

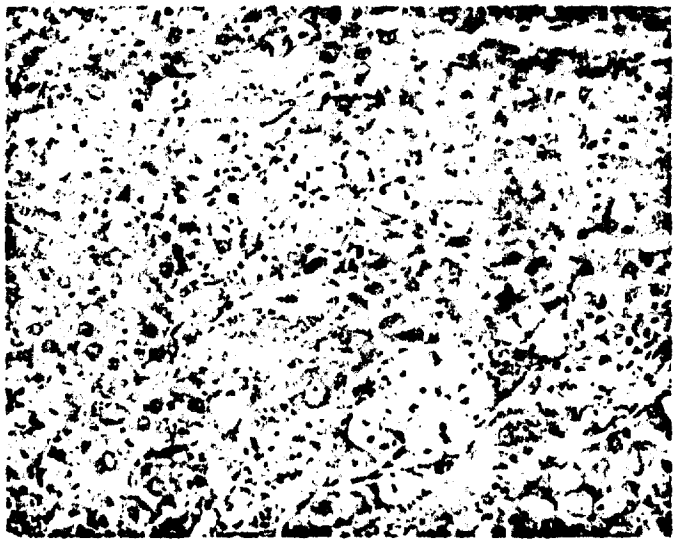

Fig. 25. — Proliferated Kupffer cells with swollen, foamy cytoplasm of a mouse (dextran).

The storage phenomena were in general distinctly less frequent and less marked in animals treated with dextran than in those given PVP's.

(*b*) Hyperplastic and Precancerous Reactions: The hyperplastic and precancerous reactions were correspondingly also less marked and less frequent in dextran animals than in PVP animals. The spleens of some mice and of one rabbit revealed marked mononuclear or reticulum-cell proliferations obliterating the lymph follicular structure of this organ. Two mice showed, moreover, pulmonary adenomas, one of which projected above the pleural surface as a polypous formation (Fig. 26). The lung of one rabbit exhibited a large area of alveolar adenomatosis.

(*c*) Cancerous Reactions: The cancerous reactions in dextran animals resembled in histologic type and in topographical distribution those seen in PVP animals. The various types of cancers found in dextran-treated animals were endothelioma of the endocardium (Fig. 27), myeloid leukemia, reticulum-cell sarcoma of the liver or of the lymph nodes, and carcinoma or carcinosarcoma of the uterus. It is noteworthy that

Fig. 26. — Polypous adenocarcinoma of the pleura of a mouse (dextran).

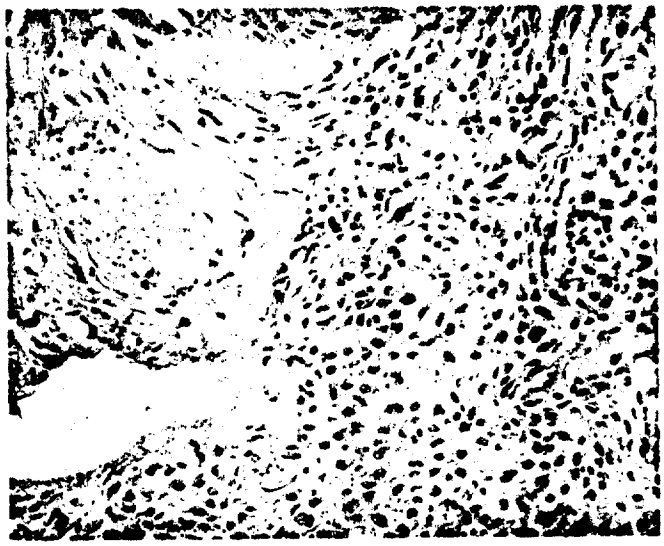

Fig. 27.—Pericardial endothelioma invading the myocardium of a rat (dextran).

this identity in histogenesis and histological structure of the cancers seen in dextran and PVP animals is shared only in part with those seen in normal control animals, which, while carrying some of the cancers recorded in the experimental animals, show them at a considerably lower incidence rate and lack also the various and frequent developmental and "precancerous" stages seen in the test animals.

IV. Silastic Rubber.—This observation applies also to the cancers found in the internal organs of rats which received implants of silastic rubber or gum into the subcutaneous tissue or into the abdominal cavity. These tumors occurred in these experimental animals at a normal frequency rate. The spindle-cell sarcomas which developed around the subcutaneously implanted cubes of silastic rubber, on the other hand, represent specific reaction products to the processed silicon polymer (Fig. 28). The histologic examination of the capsules surrounding these implants in the subcutaneous tissue and in the abdominal cavity revealed that they consisted originally of oligocellular hyaline membranes which were thicker for implants in the subcutaneous tissue than for those in the abdominal cavity. In some capsules multicentric small cellular foci were found beneath the inner lining which were not in direct contact with the implant. It may be assumed that the subsequent sarcoma formation started from these foci.

Fig. 28.—Fibrosarcoma surrounding a cube of Silastic in the subcutaneous tissue of a rat.

It is remarkable that none of the capsules formed around the abdominal implants developed sarcomas and that such an event was observed in only one of two series of 30 rats each which received implants of the silicon latex. While tumors were not formed around the implants of powdered silica placed in the subcutaneous tissue and into the peritoneal cavity, the relatively large number of cancers of the internal organs observed in rats of these series creates the suspicion that the two phenomena may not entirely be unrelated.

Mention may finally be made of the fact, which is apparent from the descriptions of the histological changes observed in the different experiments, that storage phenomena and hyperplastic reactions were present in animals with and without neoplastic reactions. In these respects animals with tumors did not differ as a group from animals without tumors.

Comment

The evidence presented indicates that under the experimental conditions observed some, but not all, PVPs; some, but not all dextrans; a polyvinyl alcohol, and Silastic are carcinogenic to mice, rats, and perhaps also to rabbits, when they are introduced parenterally. The existence of fundamental differences in carcinogenic properties between polymers of the same type, such as PVPs, was suggested by the divergent results reported previously, since Hueper and Nelson and Lusky obtained sarcomas with PVP introduced into rats, while Hecht failed to do so.

The carcinogenic effect of the water soluble polymers involves several organs and tissues. It becomes manifest mainly in organs in which these macromolecular substances are retained and stored over prolonged periods and where they tend to elicit reactive cellular proliferations which seem to supply the preparatory state for the subsequent development of cancers. In contrast, the carcinogenic effect of water insoluble polymers whenever it is present is limited to the site of implantation, i. e., mainly the subcutaneous connective tissue.

Differences in carcinogenic potency between polymers of the same type and of different types seem to be reflected in variations in the length of the minimal latent period. The minimal preparatory period of cancers for PVI's 1, 2, 3, and 4 was six to eight months. Since about 25% of all cancers among the rats of this experiment occurred during the 7th to 12th months of the exposure time, these particular PVPs exhibited a considerable degree of carcinogenic potency as judged by the length of the minimal latent period. Subsequently studied carcinogenic PVPs, dextrans, polyvinyl alcohol, and silicon rubber, on the other hand, elicited cancers not until the elapse of at least one year following the start of the experiment. A complete lack of carcinogenic response during the first 12 to 15 months of observation has been noted so far for the different polyethylene and polyurethane preparations parenterally introduced by various routes and in different physical forms.

The route of administration which apparently influences the time of onset and the degree of development of fibrous encapsulation of the implanted materials and its relative concentration in the tissues seems to exert a determining influence upon the degree of the carcinogenic effect of water-insoluble polymers, since sarcomas developed at the site of encapsulation of water insoluble polymers much more often in the subcutaneous tissue than in the peritoneal cavity. There was, on the other hand, no consistent difference in the carcinogenic response in internal organs for the various routes of introduction of water-soluble polymers.

When the degrees of carcinogenic response obtained with water-soluble polymers of different molecular size and shape were compared, again no distinct pattern of behavior was discernible. This lack of distinction extended also to reactions elicited by products of commercial grade and those of medicinal grade. The results of experiments with PVP 6 and PVP 7 which were administered in one set in single doses and in a second set in repeated doses suggested, however, that repeated exposures intensified the carcinogenic effect and thus brought to light weak carcinogenic properties which were not apparent for PVP 7 after the introduction of a single dose.

These observations are of fundamental importance because they indicate that the differences in carcinogenic behavior of different polymers of the same general type

are apparently related to much more complex differences than those represented by mere variations in average molecular weight or the ratio of branched to linear member groups in a polymer. The marked carcinogenic effect of the Silastic molecule possessing cross linkages of peroxide nature in comparison to the at best weakly carcinogenic one of the linear latex molecule provides perhaps a clue to a future study of this intricate problem. It is noteworthy in this connection that Mark,[40] working with polyvinyl macromolecules, recently pointed out that certain polymers can have different properties if they have been prepared under different conditions, not because of head-to-tail, head-to-head isomerism and not because they represent different degrees of branching, but simply because the substituents are differently arranged in space as one progresses along the chain and affect the configuration of the chain as a whole. It is, therefore, possible that the industrial use of different catalysts which determine the character of the end-groups and of different production methods in the preparation of polyvinylpyrrolidones may account for the differences observed in carcinogenic potency of PVPs of different manufacture (PVP 2 and PVP 7 of German manufacture; PVP 1 and PVP 6 of American origin).

Support of this concept of a chemospecific basis for the carcinogenic action of polymers and macromolecular condensates is provided by the recent observation of Richmond,[41] showing that an iron-dextran complex when parenterally introduced into rats was carcinogenic, while the dextran alone was not. A similar connotation may be attached to the finding that the carcinogenic polyamide "Perlon" (e-amino-caprolactam), when used as a filling of human pleural cavities, undergoes gradual disintegration of its fibrils and that in fact the fibrils varied structurally when viewed under the microscope before they were implanted. This observation indicates that at least some polymers (Mondur TD-80) do not seem to be stable in a biologic medium; nor are they chemically uniform. These facts may account for the variability of the anatomic reactions to various Perlon products, since some elicit a marked foreign-body reaction and fibroblastic proliferation, while others seem to be almost inert in this respect (Wolter).

The action of a chemical mechanism in the production of anatomic reactive lesions to polymers is, moreover, suggested by the development of granulomatous reactions in the lungs of man following the inhalation of PVP-containing hair lacquers (Bergmann, Flance, and Blumenthal) and of similar pulmonary changes in rats after the intratracheal injection of dust of Perlon and Nylon (polyhexamethylene adipamid [Massmann and Pilgrim]). The pulmonary reactions in rats were progressive and later on complicated by the development of extensive adenomatous hyperplasias originating from bronchial epithelium. Although these adenomatous responses were considered by the investigators as unrelated to the inhalation of the polyamides and although cancerous pulmonary reactions were not observed, these findings nevertheless show that some finely dispersed and powdered polymers exert distinct biologic effects in the organs of contact which must be ascribed to chemical interactions and not to the action of mere surface forces. There is no sound reason for assuming that the marked fibrosing and scar tissue development stimulating action of polyethylene films utilized in human surgical practice has any other than a chemical basis (Yeager and Cowley). The serious toxic reactions, such as alopecia, thrombocytopenia, diarrhea, and anorexia, elicited by dextran sulfate in man, are doubtlessly responses to chemical phenomena (Tudhope et al.). In fact, Tudhope compares these effects of dextran sulfate with those produced by whole-body irradiation and by exposure to mechlorethamine hydrochloride (nitrogen mustard), colchicine, and other antimitotic substances and thereby refers to chemicals and physical agents which often have ambivalent qualities, i. e., they are also carcinogenic under proper conditions.

There can be no doubt that some water-soluble as well as water-insoluble polymers are capable of producing in the organism of man and experimental animals toxic, proliferative, and even carcinogenic reactions, which in their general behavior and type cannot be distinguished from those elicited by other chemicals. The fact that evidently only some but not all polymers of the same general type seem to be carcinogenic has its well known analogue in the observation that only some and not all derivatives of certain polycyclic aromatic hydrocarbons or of aromatic amines or of azo compounds are carcinogenic and that often some specific and often apparently minor aspect of their chemical composition as well as molecular configuration are the determining factors in this respect.

Apart from a direct chemical interaction of these polymers, particularly the water-soluble variety, with cellular constituents, there exist two additional mechanisms through which these metabolically rather inert macromolecules may act upon cells. Their intracellular and extracellular storage in excessive amounts doubtlessly interferes in the long run with the normal vital cellular activities by hindering intracellular and extracellular reactions and fluid and chemical exchange processes, thereby causing cellular degeneration and death. These, in turn, may be followed by reactive regenerative cell proliferation. Such possible effects may involve the blood-brain barrier, owing to the accumulation of PVP in the choroid plexus, meninges, and adjacent cerebral tissue, and thereby impair cerebral function. They may affect the nutrition of the arterial walls as well as the liver and kidney function. Storage of PVP in the spleen, resulting in secondary reticulum-cell proliferation, may exert an influence upon erythrocytic destruction rate and thereby account for the delayed appearance of anemia often developing as a manifestation of hypersplenism in excessively stored animals (macromolecular hematic syndrome).

Since PVP has a definite affinity to form complexes with proteins, dyes, various drugs, and other chemicals, i. e., it exerts an "embathic" effect, its marked accumulation in the endometrium raises the question whether it has also an affinity to sex hormones and may elicit through the mechanism of local hyperestrogenism, endometrial hyperplasia, and carcinoma.

Concerning the merits and the validity of the theory on carcinogenesis by surface action and capsule formation of polymer films, which is also applied to the carcinogenic action of metal foils, it may be pointed out that cancers can readily be produced in rats by the implantation of certain powdered metals, such as uranium and nickel, and have been produced also in rabbits with chromium powder (Schinz). Since similar cancerous responses can also be elicited by the subcutaneous implantation of certain metal compounds, such as calcium chromate, in rats or by the intravenous injection of beryllium compounds into rabbits, it is very certain that the process of metal carcinogenesis does not depend on any nonspecific physical phenomenon related to surface forces but is attributable to specific chemical effects of these metallic elements and their proper biologic availability to the tissues.

It is, moreover, obvious that the carcinogenic effect of water-soluble polymers is not dependent upon the presence of a certain number of square millimeters of unbroken surface of polymer film.

The mere formation of a capsule around implanted polymer films also cannot be considered as the specific prerequisite for a subsequent cancerous development, since the balls of silicon latex subcutaneously implanted were as effectively encapsulated as the cubes of silicon rubber. Nevertheless, sarcomas mainly developed around the rubber cubes and not around the latex balls, indicating that cancerous responses in the capsule tissue depend on an additional and specific carcinogenic factor associated with the polymer. If indeed the formation of cellular scar tissue in rats would represent a fertile basis of the production of cancers, it is surprising that the chronic organizing

pneumonias not infrequently seen in rats do not seem to give rise to carcinomas or sarcomas of the lung, unless a specific carcinogenic agent is active. While at the present time a satisfactory explanation for the differences in cancerous responses to various polymers when implanted as intact film, perforated film, fabric, thread, or powder cannot be given, it is most unlikely that for the reasons given the hypothesis of Nothdurft and of Oppenheimer on polymer carcinogenesis is the correct one.

Finally, comments are in order concerning the applicability of the experimental observations to man, i. e., whether or not health and cancer hazards may result from any exposure to the growing number of synthetic and semisynthetic macromolecular substances for occupational or medicinal reasons or as the result of handling, using, or ingesting the large number of consumer goods either containing these chemicals or having been in contact with them.

The various storage phenomena produced by water-soluble polymers which have been observed not only in experimental animals but for the plasma extenders, especially PVP, also in man (Busch, Traenckner,[29] Gropper et al., Hartmann and Behrmann,[28] Wrage, Upham et al., Ravin et al.) in general have been considered as of little consequence as to any acute or chronic effects upon the function of the organs affected. This conclusion is probably correct unless highly excessive amounts of these agents, particularly PVP, are administered repeatedly over prolonged periods of time. The observations of the present experiments reported add to the list of already known thesaurismotic reactions from which possible delayed health hazards may result under exceptional conditions. These are the storage phenomena in the choroid plexus, the glia and ganglion cells of the brain, and the atheromatous and atherosclerotic arterial reactions seen in rabbits given injections of PVP and dextrans. If these observations indicate that the medicinal and cosmetic use of water-soluble polymers should not be practiced without caution and discrimination, this warning receives increased emphasis by the demonstration of carcinogenic effects elicited by both water-soluble and insoluble polymers. While there exist without any doubt justifiable and legitimate medical and surgical indications for the use of such macromolecular agents, the present uncertainty of their potential carcinogenic action in man upon parenteral or respiratory introduction demands that the calculated risk related to the disease to be treated outweighs definitely any possible delayed risk to health which may be created by a polymer administered.

Although at present no valid evidence exists that the use of such polymers in certain protheses, such as dentures and contact lenses, which have prolonged and intimate contact with mucous membranes, is actually related to an increased occurrence of cancer in the exposed tissues, it seems to be wise to conduct during the coming years carefully controlled surveys on the cancer incidence among persons thus exposed for determining the actual innocuousness of these plastic devices.

Similar considerations may well be extended to the medicinal use of ion-exchange resins (polyamine-methylene resin, carbacrylamine resin) administered by mouth for increasing the fecal excretion of sodium in cardiac edema or as an antacid in the treatment of gastric hyperacidity. Definite attention should be given in this respect also to the incorporation of macromolecular chemicals into other medicinal and cosmetic products, such as ointments, creams, and laxatives. Although the scientific character of the experimental evidence of the carcinogenesis of polymers does not justify any undue alarm concerning the use of such materials in food products, for the impregnation or coating of food containers, or for the coating or wrapping of foodstuffs, it appears to be a sound precautionary measure to keep all workers employed in the production, processing, and handling of polymers or plastics under proper medical supervision for insuring an early discovery

of any cancers possibly attributable to contact with these substances.

Conclusions

1. Certain water-soluble polymers, such as polyvinylpyrrolidone, polyvinyl alcohol, and dextran elicit upon parenteral introduction into rats and mice, and perhaps also in rabbits, sarcomas originating from organs and tissues in which these macromolecular substances are retained and stored, i. e., the reticuloendothelial tissues. Cancers from other tissues, such as uterus and skin, observed in rats may be directly or indirectly caused by these substances.

2. Polymers of the same general type but produced by different procedures and varying among each other in molecular weight and configuration differ greatly in their carcinogenic potency, i. e., some are noncarcinogenic, others weakly carcinogenic, and again others definitely carcinogenic.

3. The experiments conducted have failed so far to provide any information on the physical or chemical molecular factors which may account for these differences.

4. Experiments with silicon rubber and silicon latex showed that the rubber having a net molecule with many cross linkages possesses marked carcinogenic properties when subcutaneously implanted into rats, while the latex having a linear molecule is at best weakly carcinogenic.

5. The total evidence on hand concerning the biologic action of synthetic and semisynthetic polymers parenterally introduced into man and animals favors the view that their toxic, pharmacologic, and carcinogenic effects are mainly related to chemical properties and reactions rather than to their physical characteristics.

6. The existence of actual cancer hazards to man from water-soluble and insoluble polymers possibly associated not only with their parenteral introduction but also with their administration by the cutaneous, respiratory, and oral routes is at present problematical. The experimental evidence on hand, however, is sufficiently definite and serious for requiring distinct caution in the medicinal and cosmetic use of these products.

The various dextrans were supplied by Mr. H. S. Paine, The Dextran Corporation; Dr. H. E. Stavely, Commercial Solvent Corporation, and Dr. C. R. Rist, Starch and Dextrose Division, Bureau of Agricultural and Industrial Chemistry, Northern Regional Research Laboratory. The polyvinylpyrrolidones employed were furnished by Dr. B. M. Lanman, Schenley Laboratories, Inc.; Dr. J. Werner, General Aniline and Film Comporation; Prof. Dr. H. Oettel, Badische Anilin and Soda Fabrik; Dr. Beuchelt, Farbenfabrik Bayer, A. G., and Dr. H. Campbell, May and Baker, Ltd. Mondur TD-80 was supplied by Mr. J. H. Saunders, Mobay Chemical Company; polyethylene, by Dr. Th. Nale, Carbide and Carbon Corporation; polyvinyl alcohol, by Mr. R. H. Beeman, E. I. DuPont de Nemours and Company, and Silastic and silicone gum, by the Dow Corning Corporation.

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[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1a]]

Characterization and Classification of Dextrans from Ninety-six Strains of Bacteria[1b]

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Heretofore relatively few of the dextrans potentially available through bacterial fermentation have been prepared and characterized. The rapidly increasing significance of these polyglucosans for medical, industrial and research purposes motivated a survey of the types obtainable. We have prepared dextrans of high purity from 96 individual bacterial strains and characterized them by periodate oxidation-reaction analysis, by measurement of optical rotation, intrinsic viscosity, the concentration-dependent parameter of viscosity and infrared absorption, and by observations on solubility and gum properties. All the dextrans contained 1,6- or 1-glucosidically linked units, in percentages of 50–97, as well as 1,4-like and/or 1,3-like linked units; the lowest percentages of 1,4-like and 1,3-like linked units were within the limits of error of the periodate method, the highest percentages were about 50 and 40, respectively. On the basis of the proportions of 1,3-like links as indicated by periodate oxidation-reaction analysis, these dextrans have been grouped into 3 classes which contain (A) 0–2%, (B) 3–6% and (C) >6% 1,3-like links. Most strains yielded dextrans that could be placed in one or another of these classes; 6 strains, however, elaborated structurally heterogeneous dextrans the components of which belong to different classes. Our dextrans might not yet represent all possible classes, and reclassification of some of them might be indicated when more specific structural analyses become available.

The particular dextrans which were used initially in this country and abroad for conversion into synthetic blood-volume expanders came into their role more through force of circumstances than through known superiority for the purpose. When the clinical use of dextran was initiated in Sweden in 1944[2] and in other countries more recently, only a small number of dextrans had been reported in the chemical literature,[3-14] and not all of these had been well characterized chemically. It was quite certain, however, that these dextrans were homologous polymers of glucose with predominantly α-1,6 links. Differences had been found among dextrans from several strains of *Leuconostoc mesenteroides*[4,10,12] and from related organisms.[6,7,13] However, there was uncertainty as to whether the differences were dependent upon the microörganism or were due to the conditions for culturing the organisms,[8,9] to the methods for isolating the dextrans,[14,15] or to the degree of purity of the dextrans. The situation was clarified by a demonstration of the biological origin of pronounced differences in the structure of dextrans from 4 strains of *L. mesenteroides*.[16]

These evidences of the variation among dextrans, together with the knowledge that dextran-producing microörganisms were widely distributed and rather diverse in growth characteristics,[17,18] indicated the need for systematic chemical and physical study to determine the range of diversity of the polysaccharides from many different microörganisms of this type. Furthermore, data were needed which would make possible the selection of dextrans most suitable for specific applications in medicine, in fundamental research and in industry.

Such a survey was initiated, and reported here are our results on the isolation, purification, characterization and classification as to structural type of dextrans from 96 strains of bacteria. All except 2 of these strains produced their dextrans from sucrose; 2 *Aerobacter* strains transformed amylaceous dextrin into dextran.[19]

These dextrans have been characterized through determination of the chemical nature and proportions of glucosidic linkages present by periodate oxidation,[20] through measurement of specific rotation, viscosity and infrared absorption, and through observations on the solubility and the physical appearance of the highly hydrated gums and of their aqueous solutions. On representative types of these dextrans, structural determination by methylation analysis is being carried out,[21] as well as particle weight and other physical measurements[22] and serological studies.[23] Microbiological aspects of this survey will be reported elsewhere.[24]

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(1b) Presented at the Symposium on Microbial Polysaccharides at the 122nd national meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1952.

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TABLE I

PROPERTIES AND CLASSIFICATION OF PURIFIED DEXTRANS FROM 96 DIFFERENT STRAINS OF BACTERIA. IDENTITY AND ORIGIN OF THE STRAINS

Strain no. NRRL B-	Type AGU links, % 1,6-	1,4-like	1,3-like	Dextran [α]²⁵D (c 1) HCONH₂	1 N KOH	Dextran Viscosity, water, 25° [η]	k_1	Yield, %[a]	Solubility, water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
Class A Dextrans. 0-2% 1,3-like links														
1146	97	3	0	+214		1.245	1.07	11	+	Long	L.d.	NCIB	3356	P-2615[17]
1064	96	4	0	214		0.887	0.91	29	+ p	Tough, stringy	L.m.	CSMc	548	Type D[18]
1414	96	4	0	214		.869	.96	15	+	Short	L.m.	Isolate		
1145	96	2	2	214		1.029	.83	24	+	Long	L.d.	NCIB	3355	52[17]
512(F)[27]	95	5	0	215	+203	0.953	1.10	24	+	Long	L.m.	RGB[14]		Substrain of B-512[27]
640	95	5	0	214		1.280	1.03	14	+	Long	L.d.	ATCC	8086	22[17]; its dextran[7]
1066	95	5	0	215		0.521	0.83	11	+ p	Crumbly, F[g]	L.m.	CSMc		Subtype of type D[18]
1208	95	5	0	213		.628	1.37	17	+ p	Crumbly	L.m.	CSMc		Type D[18]
1210	95	5	0			.698	1.33	24	+	Short, rough	L.m.	CSMc		Type D[18]
1211	95	5	0	214		.843	1.30	16	+	Short, smooth	L.m.	CSMc		Type D[18]
1568	95	5	0	219		.476	1.37	15	+ p	Pasty, crumbly, F	L.m.	EJH		
1209	95	3	2	215		.603	0.87	18	+	Short, smooth	L.m.	CSMc		Type D[18]
1119	94	4	2	217		1.617	.86	7	+	Cohesive, stringy	L.m.	ATCC	8357	
1072	94	6	0	216		0.853	.93	24	+	Long	L.m.	ARS		Substrain of B-512
1198	94	6	0	215		.760	1.39	23	+	Short, F	L.m.	CSMc		Type D[18]
1212	94	6	0					16	+	Short	L.m.	CSMc		Type D[18]
1389	94	6	0	215		.848	1.39	4	+	Short	u	ALP		Similar to strain reported[28]
-I	93	7	0			.368	1.17	2	+	Short, tough				
1405	94	6	0	216		.660	1.34	9	+	Short	L.m.	Isolate		
1412	94	6	0	216		1.127	0.98	12	+	Long	L.m.	Isolate		
1413	94	6	0			0.704	1.21	15	+	Short	L.m.	Isolate		
1417	94	6	0	217		.654	1.14	17	+	Short, F	L.m.	Isolate		
1412	94	6	0	214		1.019	0.86	16	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1204	93	7	0			0.846	1.28	18	+	Crumbly	L.m.	CSMc		Type D[18]
1214	93	7	0					21	+	Short, F	L.m.	CSMc		Type D[18]
1197	92	6	2	212		.519	1.13	8	+	Floc. ppt.	L.m.	CSP	683	9[17]
1307	91	9	0	215		.952	1.08	19	+ p	Short, tough	L.m.	JMN, EJH	"B"	References 10, 29, 30
1388	91	9	0			.917	1.09	16	+	Short, tough	L.m.	RP		
1225	90	10	0	208				24	+ p	Short	A.c.	EJH		NCTC 4943, Ref. (19)
1223	90	10	0	212		.704	0.87	20	+ p	Short	A.v.	EJH		NCTC 7216, Ref. (19)
1500	90	10	0	215	204	.823	1.71	19	+ p	Short, tough	(L.m.)	CSMc		Type F[18]
1415	89	11	0	216		1.180	0.91	12	+	Stringy	L.m.	Isolate		
1196	88	10	2	215		0.890	1.22	26	+	Short	L.m.	WWC	"elai"	Ref. (31)
1499	86	14	0			.950	1.02	7	+	Stringy	L.m.	Isolate		
1383	84	16	0	217		.957	1.12	15	+ p	Short, rough	L.m.	RP		
1416	84	16	0	216		.875	0.89	17	+	Short	L.m.	Isolate		
1525	83	17	0	217		.843	0.88	24	+	Fluid, stringy	L.m.	Isolate		
1209	82	18	0	216		.857	1.03	14	+ p	Short, stiff	L.m.	RP		
[illegible]	81	19	0	218		.[illegible]	1.09	13	+ p	Short	L.m.	RP		

1396	81	19	0	217			19	+		Short, tough	L.m.	Isolate		
1429	81	19	0	216		.522	0.68	8	+		Short	L.d.	Isolate	
-I	80	20	0	214		.453	1.23	7	+		Short			
1526	79	21	0	216		.378	0.65	4	+			S.sp.	Isolate	
-I	77	23	0			.225	1.42	8	+		Short			
1397	75	25	0	219				21	+		Short	L.m.	Isolate	
1422	74	26	0	218		1.027	0.89	19	+		Short	L.m.	ERW	
1424	72	28	0	219		1.088	.75	17	+		Stringy	(L.m.)	JW	"D"	Refined Syrups and Sugars, Inc., strain "D" or 1053; derived from ATCC 6025
1402	66	34	0	220		0.925	.78	21	+		Short, F	L.m.	ERW		
1209	65	35	0	217		.913	.84	19	+		Short	L.m.	Isolate		
1298	64	36	0	223		1.025	.90	12	+	p	Short	L.m.	JMN	7 or "C"	Scrol. type A[29,30]
											Class B Dextrans. 3-6% 1,3-like links				
1193	95	2	3	+218		0.578	1.34	5	+		Short	L.d.	CSP	853	
641	94	3	3	215		1.041	1.02	17	+		Long	L.m.	ATCC	8082	
1205	94	3	3			0.865	1.22	26	+		Short, F		CSMc		Type D[18]
1387	94	3	3	217		1.418	1.04	14	+		Short	L.m.	RP		
1407	94	3	3	216		0.572	1.73	13	+		Short, cohesive	L.m.	Isolate		
1419	94	3	3	217		.815	1.45	19	+		Short, tough	L.m.	Isolate		
1400	93	3	4	220		.705	0.87	5	+		Short	L.m.	Isolate		
1401	93	3	4	215		.446	.87	8	+		Short	L.m.	Isolate		
1394	92	4	4	215		2.020	.70	6	+		Cohesive, stringy	L.m.	FWF[32]		
-I	92	5	3			1.472	1.44	1	+						
1410	91	5	4	217				7	+		Short	L.m.	Isolate		
1392	91	6	3	218		0.555	0.96	9	+		Stringy	u	Isolate		
1255	89	7	4	219		.696	1.22	18	+	p	Floc. ppt., crumbly	S.d.	AJK	L-337	Isolation[33]
1127	89	5	6	220		.945	0.87	14	+	p	Long	B.v.	AJK	L-343	May be same as previously reptd.[5]
1502	87	8	5		208	1.043	.97	7	+	p	Short	(L.m.)	CSMc		Type F[18]
1144	87	7	6		209	1.153	.72	9	+	120°	Short, tough	L.m.	NCIB	3354	
											Class C Dextrans. >6% 1,3-like links				
1120	85	0	15					9	−		Crumbly	L.m.	ATCC	8358	Type I[34]
1351	85	4	11	217		0.505	0.52	27	+		Short	S.v.	EJH		Ref. (35)
1389	85	7	8	220		1.102	1.23	21	+		Short	L.m.	RP		
1429	85	5	10		210	1.260	1.16	8	+	p	Crumbly	L.m.	CSMc		Type B[18]
1377	84	7	9	219		1.364	0.81	20	+		Long	L.m.	Svenska Sockerfabriks	AB VII-E	
1384	84	6	10	221				20	+	p	Tough	L.m.	RP		
1139	83	5	12		213	0.503	1.25	9	+	p 120°	Floc. ppt.	B.v.	AJK	L-344	Isolation[33]
1411	82	8	10	217		1.093	1.07	21	+		Short, tough	L.m.	Isolate		
1385	81	9	10	222	213	0.995	1.22	21	+	p	Crumbly	L.m.	RP		
1374	81	7	12	220		1.338	0.79	25	+		Stringy	L.m.	Benger's Ltd.		
1375	81	6	13	220		0.918	1.09	14	+		Short	L.d.	Dextran Ltd.		"Birmingham" strain[13,35]
1438	81	6	13		213	1.560[4]	0.89	4	+	120°	Floc. ppt.	L.m.	CSMc		Type B[18]
1438-A	79	7	14		213	1.458[4]	.86	9	+	120	Floc. ppt.				

TABLE I (*Continued*)

Strain no. NRRL B-	Type AGU links % 1,6-	1,4-like	1,3-like	Dextran [α]²⁵D (c 1) HCONH₂	1 N KOH	Viscosity, water, 25° [η]	k_1	Yield, %[a]	Solubility water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
1439	81	6	13	221		0.475	1.08	10	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1443	80	10	10	220		.418	0.86	18	+	Pasty	(L.m.)	CSMc		Type A[18]
1141	79	3	18	224		1.350	1.04	17	+	Tough, stringy	L.d.	NCIB	2706	63[17]
1192	78	4	18	223	210	0.910	1.33	22	+	Short, crumbly	L.m.	CSP	851	
1191	77	9	14	223		.882	1.35	19	+	Short, crumbly	L.m.	CSP	845	
1118	76	3	21		215	1.821[h]	0.74	9	−	Floc. ppt.	L.m.	ATCC	8293	
1425	74	8	18	222		1.105	.93	7	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1393	70	11	19	222		0.865	.91	19	+	Short	L.m.	Isolate		
1297	67	24	9	219	211			2	+ p, 120°	Short, rough	L.m.	JMN	5 or "A"	Ref. (10, 29, 30)
J-90A[37]	67	2	31		225				+ p, 120	Floc. ppt.	S.v.	EJH		Lancefield group H[37]
523	66	10	24		220	2.081[h]	1.51	6	−	Floc. ppt.	L.m.	C. Thom	535	
1121	65	2	33		222			7	−	Floc. ppt.	L.m.	ATCC	8359	Type II[34]
1142	63	8	29	230		0.389	1.60	6	+ p	Floc. ppt.	L.m.	NCIB	3351	Same origin as NRRL B-742
1433	63	30	7		217	2.605[h]	1.17	17	−	Crumbly	L.m.	CSMc		Type B[18]
1433-A	63	30	7		217	2.514[h]	1.22	6	−	Tough				
1431	62	29	9		217	3.107[h]	0.34	10	−	Floc. ppt.	L.m.	CSMc		Type B[18]
1149	52	8	40		232	2.716[h]	1.24	4	−	Fine ppt.	L.m.	NCIB	6109	
						Structurally heterogeneous dextrans and/or their major components								
742	67	21	12	+223		0.296	1.35	15.0	+ p	Short, dense	L.m.	CSP[16]	681	5[17]; 4[4]
-L	81	19	0	212		.152	1.38	(35)[i]	+ p	Fine ppt.				
-S	57	17	26	226		.326	1.45	(39)	+ p	Fine ppt.				
1254	90	7	3	216		.488	1.39	12	+ p	Floc. ppt.	S.d.	AJK	L-336	Isolation[33]
-L	69	31	0	213		.189	1.29	(7)	+ p	Floc. ppt.				
-S	93	7	0	214		.537	1.26	(55)	+ p	Floc. ppt.				
1299-L	58	36	6		+216	.873	1.05	(55)	+ p 120°	Floc. ppt.	L.m.	JMN 8 or "K"		From AJK, 1940. Serol. type A[29,30]
-S	50	50	0	221	212	.469	1.53	(23)	+	Fine ppt.				
1355-L	88	9	3		206	1.115	1.13	(37)	−	Short	L.m.	RP		
-S	57	8	35	233	220	0.193	1.24	(48)	+	Fine ppt.				
1498-A	91	9	0	212		1.136	1.81	5	+ p	Short, tough	L.m.	CSMc		Type F[18]
-L	94	6	0	213		1.096	1.30	14	+	Short				
-S	62	11	27	227		0.329	1.25	3	+	Fine ppt.				
1501-A	80	18	2	211		1.004	1.67	7	+ p	Short, dense	L.m.	CSMc		Type F[18]
-L	93	7	0		206	1.054	1.34	7	+	Short				
-S	65	15	20		216	0.412	1.28	5	+	Fine ppt.				

[a] Based on weight of sucrose in culture. [b] +, soluble; −, insoluble; p, if precautions are observed; 120°, solution completed by autoclaving. [c] Observed when precipitated from aqueous solution by ethanol of 45–50% concentration. Products are gums unless otherwise stated. [d] Identities are as confirmed or determined[24] except for those indicated in parentheses, which are as received. A, *Acetobacter;* B.v., *Betabacterium vermiforme;* c, *capsulatum;* d, *dextranicum;* L, *Leuconostoc;* m, *mesenteroides;* S.d., *Streptobacterium dextranicum;* S.sp., *Streptococcus* species; S.v., *Streptococcus viridans;* v, *viscosum;* u, unidentified. [e] NCIB, National Collection of Industrial Bacteria; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures. [f] Initials stand for names of donors, as follows: R. G. Benedict; W. W. Carlson; E. J. Hehre; G. J. Hucker; A. J. Kluyver, C. S. McCleskey; J. M. Neill; R. Patrick; C. S. Pederson; A. L. Pollard; A. R. Stanley; J. Warren and E. R. Wolford. [g] F, fluorescent in ordinary light as prepared; type [illegible]. [h] 5% or 1% potassium hydroxide. [i] Values in parentheses are per cent. of the purified fraction obtained from the whole dextran.[37]

Experimental

Cultures.—The dextran-producing bacteria employed in this survey were obtained from several culture collections, from other investigators of these microörganisms and of their polysaccharides and by isolation from various natural sources. Each of these pure cultures, which is made up of descendents of a single isolation, is defined as a "strain."[25] Pertinent data are given for each strain in Table I. Although we designate the strains and their respective dextrans by the NRRL number of the strain in the Culture Collection of the Northern Utilization Research Branch, we cite previous designations of cultures as well as publications concerning them or their dextrans.

With a few exceptions, the strains in Table I are believed to be free of duplication; such cultures as originated from a common natural source showed differences in taxonomy or in the physical and chemical characteristics of their dextrans. A few strains which had a common origin but different subsequent history have been retained purposely as separate strains because their dextrans differed significantly (B-742, -1142; B-1424, -1297) or their total fermentation products are of special interest (B-512, -1072).[26]

Dextran Production.—Most of the dextrans reported here were prepared under standardized culture conditions. The set of conditions used could not be expected to be optimal for each organism and doubtless caused yields and viscosities of dextrans from certain strains to be lower than might have been obtained under conditions more adequately fulfilling the specific requirements of those strains. For example, the yield of B-1149 dextran can be increased by inclusion of supplementary vitamins in the medium, and the viscosity of B-512 dextran is decreased sharply when the incubation time is extended, as in this survey, beyond that required for completion of dextran production.[14] However, nearly every organism has been cultured for dextran production under from 2 to 9 different conditions without change in the type of dextran being detectable by our methods.[27] Therefore, reproduction of the dextran preparations reported here can be expected under our conditions of culture propagation and medium composition. It cannot be assumed, though, that the dextran products would remain unchanged under all possible modifications of culture conditions.

The dextrans were isolated from liter cultures after incubation at 25°, usually for 5 days, in a sucrose, tryptone, phosphate medium supplemented with yeast extract, liver extract or malt extract. Essentially anaerobic conditions were encouraged by "deep" culturing. Specific details on the culturing of the organisms, the conditions for dextran production, and the relation of these conditions to the dextran product are to be reported elsewhere.[24]

Special conditions were established for production of dextran from B-1225, -1226 and -1351.[19,28] Dextran from strain F90A, which was the product previously described,[29] was given further purification before being characterized by our methods.

Observations on Cultures.—At the end of the period of dextran production, the cultures presented a variety of appearances. Usually there was no pronounced odor; the *p*H values usually were 4 to 5. The cultures from some strains showed marked fluorescence in ordinary light, as indicated in Table I, and as reported previously by others.[18] Almost invariably the cultures were cloudy or opaque. The gross viscosity of cultures from different strains ranged from almost solid gels and thick fluids to thin fluids, and usually was proportional to the yield of dextran present. Many of the viscous cultures were dull and turbid (notably B-1254, -1255 and -1308); others were glistening and semitransparent (B-512, -1145, -1146, -640, -641 and -1412). Still other cultures appeared to have a gum phase uniformly dispersed throughout the medium (B-1119, -1394 and -1433). In many cases gum- or gel-like material had separated out on the bottom of the culture flask (notably B-1254, -1255 and -1394, as well as strains requiring extended periods of incubation). Often this second phase appeared to dissolve in the culture when stirred or shaken. In some cases mixing was avoided[26] and this second phase was isolated and purified separately giving the "I" fractions of B-1380, -1394, -1420 and -1526. From cultures that showed flocculent particles suspended in either thin or viscous solutions (B-523, -1118, -1149 and others), water-insoluble dextrans were obtained.

Isolation and Purification of Dextrans. (A). Water-soluble Dextrans.—The cultures were made to 33–35% by volume with ethanol[30]; viscous cultures first were blended with water and diluted to 2 volumes or less with water. These alcoholic solutions were slowly passed twice through a continuous supercentrifuge. This removed bacterial cells, insoluble matter and dextran fractions of low solubility. The concentration of ethanol in the supercentrifugate then was increased just to the point where dextran precipitation appeared complete (usually 42–45% ethanol) or else to 50% ethanol. The supernatant fluid was decanted promptly from gummy products or centrifuged from flocculent precipitates. By kneading or stirring with 50% ethanol and then reprecipitating from water solution 3 successive times by addition of an equal volume of ethanol,[14] the dextran product was purified from adhering nutrients from the medium and from by-products of bacterial fermentation. All of these operations were carried out at room temperature.

For reprecipitations, the dextran products were dispersed most successfully by gradual addition of water and stirring to obtain homogeneous pastes before diluting further. Some products appeared to become less soluble during purification, especially in the presence of 50% ethanol, and required autoclaving at *p*H 5–6 to obtain in about 5% aqueous solution (some B-1254 preparations, B-1431 and -1433). Dextran products readily soluble in water were dehydrated by adding the aqueous solution to absolute ethanol, washing with ethanol and finally drying under anhydrous conditions as previously described.[14] Dextrans less readily soluble (such as B-1139, -1144, -1193, -1299L, -1355L and -1433) were dehydrated in the frozen state under high vacuum (lyophilized) to insure greater ease of dissolution later.

Description will be given elsewhere of the methods used in separating and purifying the polysaccharide fractions insoluble at ethanol concentration of 35% (for example, fractions B-1458-A, -1433-A, -1498-A and -1501-A), as well as those requiring 55% or higher concentrations for precipitation.[24]

(B) Water-insoluble Dextrans.—Cultures that showed much insoluble gum or flocculent particles after dilution and vigorous agitation were treated at 25° with 10% potassium hydroxide solution to give a final concentration of 1 *N*. After supercentrifugation, the *p*H was adjusted to 3–4 with acetic acid and the dextran precipitated with ethanol. The product was washed with 50% ethanol and thrice reprecipitated from aqueous solution. Often, potassium hydroxide was necessary for redissolving the dextran in the course of purification (examples: B-523, -1118, -1120 and -1149); such dextrans were given a final precipitation from water suspension to reduce salt content. In other cases, autoclaving the dextran at *p*H 5–6 produced solution (examples: B-1431 and -1433).

(25) R. E. Buchanan, R. St. John-Brooks and R. S. Breed, *J. Bacteriol.*, **55**, 293 (1948).

(26) C. A. Wilham, B. H. Alexander and Allene Jeanes, in preparation for publication.

(27) In 1950, the B-512(F) substrain supplanted B 512 for all work at the Northern Utilization Research Branch. Since that time, the dextran from this substrain has been designated inexactly as B-512 in numerous publications, and will be so designated hereinafter in this paper. The dextrans from B-512 and B 512(F) appear to be identical.

(28) P. E. Smith and A. L. Pollard, *J. Bacteriol.*, **63**, 123 (1952).

(29) J. M. Neill, J. V. Sugg, E. J. Hehre and E. Jaffe, *Proc. Soc. Exptl. Biol. Med.*, **47**, 339 (1941).

(30) E. J. Hehre, *ibid.*, **54**, 18 (1943).

(31) W. W. Carlson and Virginia Whiteside-Carlson, *ibid.*, **71**, 416 (1949).

(32) F. W. Fabian and R. H. Henderson, *Food Research*, **15**, 415 (1950).

(33) L. B. C. Perquin, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **6**, 257 (1939-1940).

(34) J. A. Alford and C. S. McCleskey, *Proc. La. Acad. Sci.*, **6**, 36 (1943).

(35) E. J. Hehre, *Bacteriol. Proc.*, 23 (1952).

(36) W. N. Haworth and M. Stacey, Brit. Patent 613,909 (March 2, 1949).

(37) For this dextran, we are indebted to Drs. E. J. Hehre and J. M. Neill, who described its preparation and partial characterization in *J. Exptl. Med.*, **85**, 147 (1946).

(38) E. J. Hehre and J. M. Teichlyz, unpublished results.

(39) All ethanol concentrations are in terms of absolute alcohol.

These dextrans were dehydrated from aqueous dispersions (*p*H of 5–6) by lyophilization.

(C). Heterogeneous Dextrans.—Some dextran products after precipitation from the supercentrifugate by ethanol in the concentration range 35–42 or 35–45%, were found to be separable into fractions having distinctly different properties.[26] In some cases the whole dextran was characterized (B-742 and -1254; Table I, heterogeneous group), as well as its less soluble and more soluble components, designated by the suffixes -L and -S, respectively. In other cases, only the components fractionated from the dextran were characterized (B-1299, -1355, -1498 and -1501). Complete details on the methods and all the products of fractionation will be reported elsewhere.[26]

Analytical Methods. (A). Moisture.—After completion of drying, all dextrans were equilibrated with atmospheric moisture under constant conditions of 21° and 61% relative humidity. The dextrans were stored, and all samples for analyses were weighed, under these same conditions. For determination of moisture content, approximately 0.3-g. samples were held *in vacuo* in an unheated oven for about 16 hours and then heated at 100° and 2 mm. pressure to constant weight (about 30 hours). Moisture contents usually were 14–16%. Dextrans heated in this way have been reported to retain about 0.30% moisture, as shown by use of the Karl Fischer reagent.[40]

All calculations for other analyses were made on a dry basis.

(B). Periodate Oxidation.—The types and proportions of anhydroglucopyranosidically linked units (AGU) in the purified dextrans were determined by sodium metaperiodate oxidation.[16,20] Units designated as linked 1- or 1,6-, 1,4-like or 1,2-like reduced 2, 1 or 0 moles of periodate and produced 1, 0 or 0 mole formic acid/mole AGU, respectively. All values reported for water-soluble dextrans are for the 72-hour period of oxidation. Measurements of periodate reduced were made at 25° unless stated otherwise.[20]

Formic acid measurements were precise to ±0.001 mole/mole AGU and accurate to within 1%. The calculation of non-1,6-linked units is based upon measurement of periodate reduced. Measurements made at 25°, such as those in Table I, were precise to ±0.02 mole IO_4^-/mole AGU. However, the percentages of 1,4-like and 1,3-like linked units reported in Table I may be in error by as much as 5%. When the measurements were made at 4°, the error was reduced to 2–3%.[20] This statement of accuracy is based on methylation-structure analysis of the one dextran, B-512, which shows it to have 95% 1,6-linked units and 5% 1,3.[21] Under the conditions of periodate oxidation–reaction analysis by which the data shown in Table I were obtained, this dextran appeared to have 5% 1,4-like linked units and no 1,3-like.

(C). Specific Rotation.—Specific rotations were read with sodium vapor light, on solutions filtered through fritted glass when necessary to remove traces of extraneous matter, and are accurate to ±2°. Dextrans were dissolved in ice-cold formamide which had been distilled *in vacuo*. Those of low solubility in water were insoluble in formamide and were dissolved either in ice-cold potassium hydroxide (1 *N*) or were autoclaved in a small amount of water to increase hydration and then made up to 1 *N* with potassium hydroxide solution. Only a few dextrans gave sufficiently clear solutions in water to permit measurement of rotation (examples: B-512, -1072, -1127, -1197).

(D) Intrinsic Viscosity and k_1 Parameter.[41]—For measurement of intrinsic viscosity, water-soluble dextrans were dissolved at 4 or 25° and then autoclaved at 15 lb./in.² (120°) for 30 minutes (*p*H 5–6). Water-insoluble dextrans were dissolved in 1 *N* potassium hydroxide solution. Measurements were made on the original solution after pressure filtration through fritted glass and on two other solutions obtained by successive dilutions of the filtered original. These dilutions were made gravimetrically. Measurements were made in No. 100 Ostwald–Cannon–Fenske tubes. The k_1 parameter was calculated from the concentration dependence of the specific viscosity.[42]

(E) Other.—Nitrogen analyses were made by the micro-Kjeldahl procedure on 100-mg. samples; phosphorus was determined by a colorimetric method.

Qualitative tests for fructose in dextrans were made by use of 85% phosphoric acid to a limiting value of about 0.2%.[14] Quantitative measurement was made by colorimetric methods.[43]

Results

Data on the purified dextran products and on the strains from which they were derived are shown in Table I.

In Table I, the dextrans are organized into three classes (A, B and C) in which they are arranged in the order of decreasing contents of 1,6-linkages, and increasing contents of 1,4-like and 1,3-like linkages. Structurally heterogeneous dextrans which contained components belonging in different classes are listed separately at the end of Table I.

Products Included in Table I.—With few exceptions the dextrans in Table I comprise major parts of the total fermentation products of the cultures precipitated from the culture supercentrifugates by ethanol in the concentration range 35–50%. These dextrans are designated merely by the strain number, *e.g.*, B-1146. The six structurally heterogeneous dextrans have been subfractionated; in each case data are shown for the less soluble (L) and the more soluble (S) components. A few representatives of the other types of polysaccharide products also obtained from the dextran-containing cultures are included in Table I and are designated by suffixes to the strain number ("I," not in solution in the culture but water-soluble during purification; "A," soluble in the culture but insoluble in the range of ethanol concentration, 0–35%). "A" fractions were always removed from the culture but were not always isolated; only a few of those isolated are reported here. The presence within the same culture of fractions which have the general characteristics of dextrans but which differ physically and/or chemically evidences the molecular heterogeneity of dextran preparations. The phenomenon of molecular heterogeneity of dextran will be discussed more completely elsewhere.[26]

Yields.—The highest yield of purified dextran product, based on the weight of sucrose in the culture, was 29%. This is 61% of the theoretical. Losses during purification usually were about 3–5%. The average yield of the products listed in classes A, B and C were 16, 11 and 13%, respectively.

Our objective was to obtain representative dextrans of highest purity, rather than to develop conditions for maximal yields. Attention to individual culture requirements doubtless would improve the yield from many strains.

Composition of Purified Dextrans.—The purified dextran products contained no more than about 0.02% fructose, which is the limiting value of the colorimetric method of analysis employed.[43] Dextran B-1351 was the sole exception[44]; it contained 0.26% fructose which has been shown to be a constituent of the dextran.[44]

Glucose was the only sugar found by paper chromatography of a few dextrans of special interest.

(40) R. L. Weiman, R. H. Cundiff, T. C. Yao, E. E. Toops, Jr., and J. A. Riddick, *Abstracts Papers Am. Chem. Soc.*, **122**, 15A (1952).

(41) We are indebted to Dr. N. N. Hellman for planning the procedures for measurement of intrinsic viscosity and for calculation of k_1 values.

(42) (a) K. Sinha, *J. Research Natl. Bur. Standards*, **42**, 403 (1949); (b) *J. Colloid Sci.*, **5**, 386 (1950).

(43) C. S. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, *Abstracts Papers Am. Chem. Soc.*, **124**, 4D (1953); *Anal. Chem.*, in press.

(44) E. J. Hehre, private communication.

est (B-512, -523 and -742) after complete acid hydrolysis.[45]

Percentages of nitrogen, phosphorus and ash did not exceed 0.01, 0.003 and 0.05, respectively, and often were less in both water-soluble and water-insoluble dextrans.

Character of Dextran Precipitates and Solutions.—The nature of the dextran product was observed after precipitation of the dextran from aqueous solution by ethanol of 45–50% concentration and during washing with 50% ethanol (Table I). The products were either gums, or flocculent or fine powders. The gums were either "long" (that is, under gentle tension they showed elasticity or ability to flow rather than breaking apart) or "short" (that is, under slight tension the gum broke apart readily). All gradations of these types were found. Thus, the "stringy" gums pulled out to fine threads but showed less tendency to stream in large masses than did the "long" gums. Some of the stringy gums were fluid or sirupy.

The dextrans were isolated in such ways that molecular aggregation did not contribute significantly to insolubility.[15] The ease of solubility of the dextran was, therefore, a characterizing property. Dissolution of dextrans in water, formamide or dilute alkali occurred much more readily at about 4° than at 25°. Heating dextrans of low solubility usually was ineffective unless dispersion already was essentially homogeneous. Many dextrans in classes A and B dissolved readily in concentrations up to 30–40% merely by adding water (examples: B-1146, -512, -1307 and -641). Other dextrans appeared to be insoluble unless water was worked in gradually to allow all particles to become dispersed in their own dense paste (examples: B-1064, -1066, -1382, -1383, -1308 and -1255). The majority of dextrans in class C and in the heterogeneous group were much more difficult to dissolve than those of class A and became progressively more so as the content of non-1,6-linkages increased.

The dense (40–50%) aqueous dispersions of most dextrans were brilliantly clear; some notable exceptions were dextrans B-1196, -1405, -1407, -1414 and -1419. Dilute aqueous solutions (2–5%) of the long and stringy gums were clear or slightly opalescent. With a few exceptions all others were turbid to varying degrees.

Discussion

General Aspects of Survey.—The 96 bacterial strains used for production of the dextrans described here came from 5 genera and constitute the individual strains from a total of 135 examined. Although the strains were from a variety of natural sources,[24] the sampling was not sufficiently large or diverse to assure inclusion of all possible dextran-producing types. Sufficient correlation has not been found between strain classification or origin and dextran characteristics to provide a basis for classification of the dextrans.

The assumption may be made with reasonable assurance that the one constant feature of all these dextrans is their structural component which appears to be almost exclusively the anhydroglucopyranose unit of alpha configuration. The other chemical and the physical characteristics of the dextrans cover wide ranges of values, as is shown in Table I. The 1,6-glucosidic linkages constitute from 50–97% of the total linkages. As determined by periodate oxidation–reaction analysis, the non-1,6-linkages are of two types, the 1,4-like and the 1,3-like. Either type may constitute all or only part of the total non-1,6-links. The intrinsic viscosity, solubility and nature of the dextran precipitates, like all the other dextran properties, constitute continuous spectra of values having small increments of variation, yet differing widely in the extremes. However, definite natural types among the dextrans are indicated by the recurrence of certain combinations of properties in dextrans from different strains.

Classification of Dextrans.—From the different types and proportions of glucopyranosidically linked units present in the various dextrans, as determined by periodate oxidation, it is clear that a great range of structures is represented in the dextran class of polysaccharides. A convenient separation of the dextrans into three classes has, nevertheless, been made using the periodate oxidation–reaction data (Table II). The differentiation of these classes depends primarily upon the content of 1,3-like linked units. The demarcation in content of 1,3-like linked units for class A was based upon the limit of positive detection, 2% instead of 0%. For class B, the actual proportions of the 3 types of links in the dextrans determined the point of division. Class C dextrans, with a few exceptions, had significantly higher contents of 1,3-like links or lower contents of 1,6- than those of class B. Those dextrans found to be separable into components belonging in different classes have been grouped separately. Data on these dextrans and/or their major components are shown at the end of Table I. Although at present, dextrans from only 6 strains have been shown to be structurally heterogeneous, we believe that further investigation of a number of other dextrans (such as B-1112, -1192, -1255, -1351 and -1374) would reveal a similar complexity.

TABLE II

CLASSIFICATION OF DEXTRANS

	Carbon atoms of AGU involved in glucopyranosidic linkages: C_1 (C_1 (or C_1 and C_6))	C_1 (or C_1 and C_6) and C_6-like	C_1 (or C_1 and C_6) and C_3-like	No. of dextrans
	Designation[a] and percentage of linkages			
Class	1,6	1,4-like	1,3-like	
A	97–50	0–50	0–2	47
B	95–86	0–8	3–6	15
C	85–50	0–36	>6	28
	Molecularly heterogeneous dextrans which have been fractionated into major components belonging in 2 or more of the other classes, respectively.			6

[a] The significance of the term, "like," has been stated previously.[20]

The lowest value for 1,4-like linked units in dextrans of classes A and B, 2% (Table I), is within the precision of our analytical procedure. Furthermore, as has already been shown for B-512 dextran, other dextrans in these classes might appear by periodate oxidation procedures to have higher contents of

(45) R. J. Dimler, H. A. Davis, G. J. Gill and C. E. Rist, unpublished data.

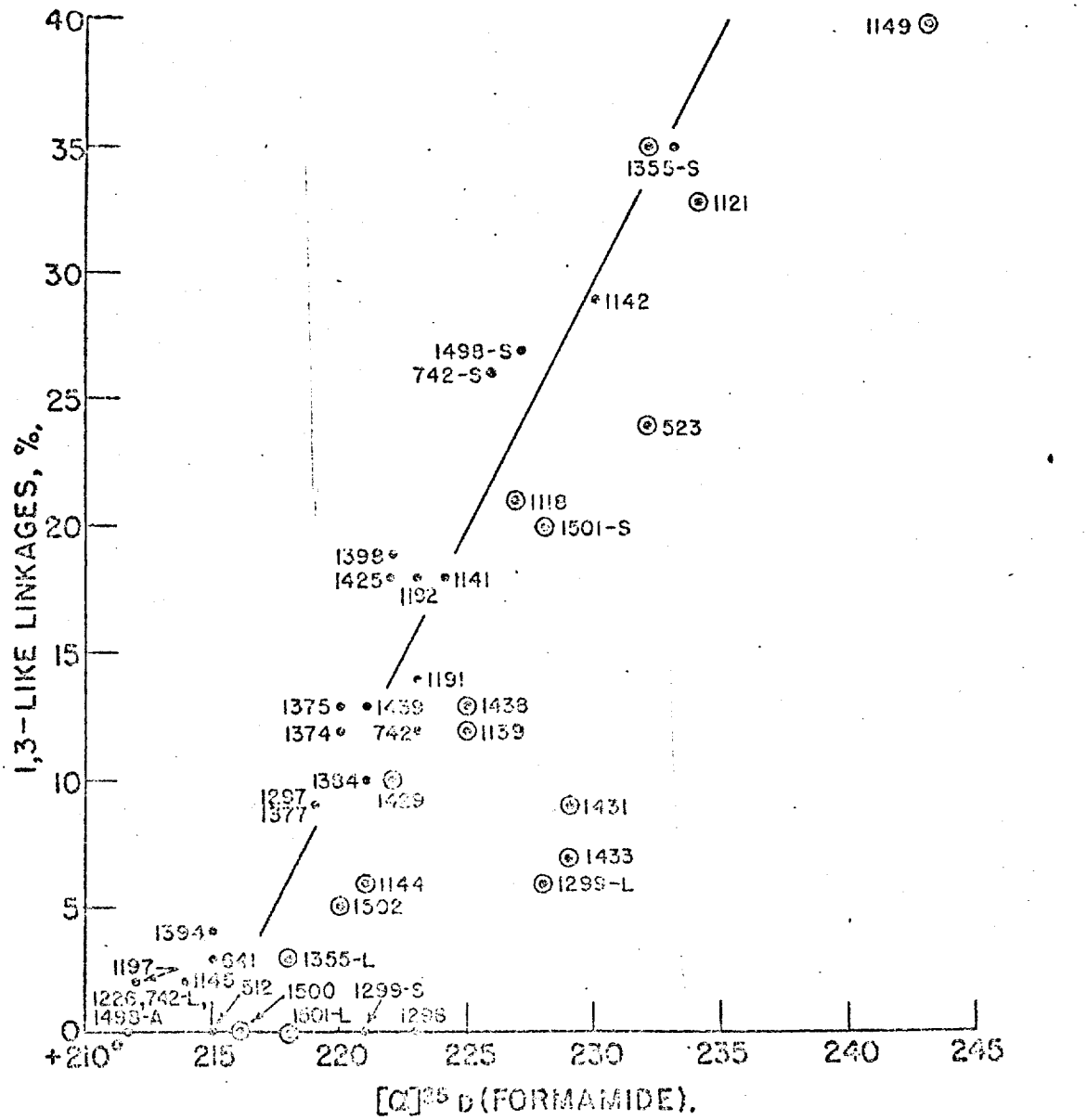

Fig. 1.—Correlation between specific rotation and content of 1,3-like linkages in dextrans: ⊙, rotation observed in formamide; ⊙, rotation observed in 1 *N* potassium hydroxide and calculated to formamide by assuming that all dextrans show the same difference in rotation in these solvents as do dextrans B-512 and B-1355S.

these links than are actually present. Therefore, the lower limit for 1,4-like links is indicated as 0% in Table II.

This mode of classification, although restricted by the limitations of the periodate oxidation method of structural analysis,[20] is adequate for the present and permits extension in the future. Specific identification of the non-1,6-linkages will permit more accurate classification of the dextrans. As will be shown in a later section, the type of link designated 1,3-like already appears to include more than one kind of structure. The same might also be found true for the 1,4-like type.

Correlation among Dextran Properties

Linkages.—The arrangement of the dextrans within the classes in the order of decreasing content of 1,6-links and increasing content of the non-1,6 is suggestive of an order of increasing degree of branching. However, the periodate data provide no direct evidence for this interpretation. Non-reducing end groups are not differentiated from units within the chain linked through C_1 and C_6. The non-1,6-linked units merely indicate the maximum proportion of branch points possible, but some or all of them may be units within a main, straight chain. However, methylation data for B-512 dextran[21] and for several other dextrans,[13,15] prove the non-1,6-linked units to constitute branch points.

Specific Rotation.—The specific rotation of dextrans differs with the solvent.[16] Thus, B-512 dextran showed values of 199, 203 and 215° in water, 1 *N* potassium hydroxide and formamide, respectively. Rotations in formamide ranged from 208

(16) All rotations referred to are positive and were obtained in formamide unless stated otherwise.

to 233° for the different soluble dextrans, and from 203 to 232° for those dextrans tested in potassium hydroxide. Though not recorded in Table I, rotations in water were measured for a few dextrans, with 196° the lowest value observed. None of our dextrans had specific rotations near those of 180° in water[8,10,13] and 180–190° in alkali[6,9,10,13] reported for some dextran preparations.

The rotation, 215 ± 2°,[46] was characteristic of the great majority of class A dextrans which had more than 75% 1,6-linkages. Thus, variation of 1,6-linkage content between 97 and 76% and of the 1,4-like between 2 and 24% did not appear to influence the rotation. This might have been expected from the fact that the specific rotation of starch in water, +200°, is essentially the same as that for B-512 dextran. Dextrans of classes B and C had no characteristic rotation, but usually the rotation increased proportionally with the content of 1,3-like linked units as is shown in Fig. 1.

However, class A dextrans having 1,4-like linkages ≧25% (excepting B-1399 dextran) showed rotations significantly higher than 215°. This was true also of the similarly constituted fractions from B-1299 (heterogeneous group) (Fig. 1). It appears that the high rotations of these dextrans and dextran fractions are related to the presence of 1,3-like linked units which, as is shown in the following section, have been detected by infrared absorption analysis but not by periodate oxidation. There is good agreement between the rotations and the contents of 1,3-like linked units estimated from the infrared spectra of all these preparations except B-1298 and B-1299L. For these two substances, the rotations are so high as to suggest the influence of some additional and unidentified factor.

Rotations significantly lower than 215° were shown by a few class A dextrans. Dextran B-1197, with rotations 212 and 196° (water), is known to contain some 1,3-like linked units but no detected fructose. Dextrans B-1225 and B-1226, which were produced from dextrin by *Acetobacter* species, showed rotations of 208 and 212°, respectively. Our rotation for the B-1225 dextran is in agreement with that previously reported by Hehre.[15b] This dextran, as well as the dextran fraction, B-742L (heterogeneous group) which also showed a rotation of 212°, was outstandingly free of 1,3-like linked units. Absence of this type of link might also account for the rotations, 211–212°, shown by several other dextran fractions.

Infrared Spectra.—Infrared absorption analysis provides further evidence of the presence of a distinctive structure in dextrans which show 1,3-like links by periodate analysis and/or rotations higher than about 212 ± 2°. Dextrans were differentiated by Burket and Melvin as "Type I" and "Type II" according to whether they showed little, or appreciable, absorption at 12.6 μ.[47] From the infrared absorption spectra of dextrans, Melvin, *et al.*, now have calculated the percentage of Type II structure present[48] and from these values the percentages of 1,3-like links in the dextrans have been estimated. These procedures utilized the facts that our dextran preparations, B-742L, which shows no 1,3-like linked units by periodate analysis, and B-1355S, which shows the highest content of these links by periodate analysis of any of our products, are models of the infrared Types I and II, respectively.

In Table III are shown some representative values of 1,3-like linked units obtained by periodate oxidation and by infrared analysis. These values establish direct, quantitative relationship between the contents of Type II and of 1,3-like linked units in most dextrans.

TABLE III

CONTENT OF 1,3-LIKE LINKED UNITS IN DEXTRANS, CALCULATED FROM PERIODATE OXIDATION AND FROM INFRARED ABSORPTION DATA

Dextran NRRL B-		1,3-Like links, %	
		Periodate[a]	Infrared, Type II
A	1146	0 (3)	3
	512	0 (3)	5
	1308	0 (2)	3
	1197	2	2
	1225	0	0
	1383	0 (0)	4
	1382	0 (1)	2
	1424	0 (0)	5
	1402	0 (0)	5
	1298	0 (0)	6
B	1193	3	4
	641	3	2
	1387	3	4
	1419	3	5
	1255	4	4
C	1351	11	11
	1139	12	9
	1385	10 (13)	14
	1118[b]	21	5
	1192	18	18
	1191	14	18
	523[b]	24	2
	1121[b]	33	11
	1432[b]	7	5
	1149[b]	40	0
Heterogeneous-dextran group			
	742L	0 (0)	0
	742S	26	27
	1299L	6	7
	1299S	0 (0)	9
	1355S	35	35

[a] Values in parentheses were obtained by making the iodimetric titration for periodate reduced at 4° instead of at 25°.[20] [b] This is one of the dextrans which showed enhanced infrared absorption at 10.6 and 12.2 μ.

Most of the class A dextrans listed in Table III are ones whose percentages of 1,3-like linked units as determined by periodate analysis appeared low; others not listed also showed detectable Type II absorption. For class A dextrans having more than 75% 1,6-linked units, agreement between periodate and infrared analyses was within the precision of the methods, especially if titration for periodate was made at 4°[20] (Table III). For most class A dextrans having 75% or less 1,6-links (B-1397, -1424, -1402 and -1298) the differences were greater

[47] S. C. Burket and E. H. Melvin, *Science*, **115**, 519 (1952).
[48] E. H. Melvin, *et al.*, data in preparation for publication.

and were not eliminated by 4° titration. This is true also of dextran fraction B-1299S, but not true of B-1299L (heterogeneous group).

With a few exceptions, the dextrans of class C and the heterogeneous group showed good agreement between contents of 1,3-like linked units as shown by periodate and by infrared analyses (Table III). The main exception was the water-insoluble dextran, B-1149, which showed no Type II absorption, but slightly greater absorption at 10.6 and 12.2 μ than did dextrans of Types I and II. Several other water-insoluble dextrans (B-1121, -523, -1118, -1433 and -1431) showed this new absorption in decreasing amounts and in addition to some Type II absorption (Table III). The rotation of most of these dextrans was higher than would be expected from periodate analyses (see Fig. 1).

The correlation among data from periodate oxidation, optical rotation and infrared absorption appears to indicate that in most dextrans the 1,3-like links are identical; possibly they are the 1,3. However, a different structure not distinguishable from the 1,3 by periodate oxidation appears to be present in a few dextrans.

Intrinsic Viscosity.—The intrinsic viscosities[49] show a wide range of values in each class and an over-all range of 0.15–2.0. Even the highest of these viscosities are relatively low for polymers of such high molecular weight. In both classes A and C, the maximum in the number distribution of intrinsic viscosities was in the range 0.85–1.10. In class A, there were more dextrans of viscosity below this range than above it, but the reverse was true for class C dextrans.

No consistent trend appears in the viscosity values[42] as the proportion of 1,6-linked units decreases (Table I). If it be assumed that this decrease corresponds to increased branching and that the frequently observed proportionality between linearity and intrinsic viscosity be applicable to these dextrans, a trend toward lower viscosities should be evident. Likewise contrary to the observations, the distribution of viscosities in classes A and C would be expected to conform to this proportionality between linearity and viscosity. However, the influence of branching might be obscured by variations in particle weights.

There appears to be rough proportionality between the content of 1,6-links and viscosity in the series of fractions from dextrans B-1254, -1355, -1498 and -1501, respectively (Table I, heterogeneous group). However, this is not true for the B-742 fractions. The known particle weight of B-742L is so high as to preclude size as the cause for its exceptionally low viscosity.[50]

These observations permit no generalizations concerning the influence of the type and proportion of 1,6-links on the viscosity of dextrans in water.

The highest viscosities obtained were for water-insoluble dextrans in 1 *N* potassium hydroxide (B-523, -1433 and -1431; Table IC). There is insufficient information to establish a positive role for the solvent in producing these high viscosities. The fact that for a few water-soluble dextrans the intrinsic viscosities in 1 *N* potassium hydroxide normally were only 1.2–1.4 times those in water,[51] would indicate a value in water of about 2.2–1.9 for dextran B-1433. This is significantly higher than the intrinsic viscosities for any of our dextrans excepting B-1304 (class B).

Shape Factor—Gum Properties.—The concentration dependent parameter of viscosity, k_1, which is shown for the dextrans in Table I, was defined by Simha and shown theoretically to depend in a characteristic manner on particle shape, the solvent environment and molecular weight.[42] Branched molecules appear to have higher k_1 values than linear molecules.[42b] Values in the range 0.36–0.77 were derived for molecules of lowest density, such as those thread-like or randomly coiled in shape, and 0.64–2.24 for the densest or spherically shaped ones.[42]

Specific deductions concerning particle shape cannot be based on the values reported here since several factors which influence the k_1 parameter have not been measured for our dextrans. However, for this extensive series of polysaccharides, it seems justifiable to point out that the k_1 values are all within the limits calculated by Simha, and that definite relationships exist between k_1 and certain other data for the dextrans.

The k_1 values for our dextrans make up a continuous series. In this series, the dextrans do not occur randomly, but group themselves at definite positions according to their percentage of 1,6-links and to the characteristics of their gums or precipitates (Fig. 2). The boundaries of the groups encompass dextrans having comparable gums or precipitates (Table IV), and were drawn arbitrarily to show the relationships most simply. Groups have been subdivided when dextrans of class C showed k_1 values and properties comparable with those of classes A and B (groups 3a, 3b and 4a, 4c) or when dextrans within classes A and B showed significantly different properties (groups 4a, 4b and 5a, 5b).

Dextrans in groups 2, 3a, 4a and 4b show very homogeneous and distinctive properties, respectively, as well as relatively narrow ranges of intrinsic viscosity (Table IV). Group 2 is composed almost exclusively of fractions of dextrans having the lowest of all our viscosities. Dextrans of groups 5a and 4c show wide ranges in viscosity and several types of gums. Several of the dextrans in 5a had short gums, but others showed stringiness (B-1403, -1415, -1392 and especially -1127). In group 4c there are gums which were long (B-1377), stringy (B-1374 and -1424), fluid and stringy (B-1425, -1439 and -1525), as well as short. All the dextrans having long or stringy gums had k_1 values in the range 0.70–1.1, and all except one in the range 0.80–1.1.

Most of the other dextrans listed in Table I but not included in Fig. 2 also fit into one of the established groups. In several cases, lack of conformity revealed the k_1 value to have been in error. Several other values appear questionable since their dextrans showed no characteristics of the groups in which they would fall (for example, B-1414 in 4c

(49) Intrinsic viscosities in water are referred to unless stated otherwise.

(50) J. N. Hellman, private communication.

(51) J. E. Cluskey, R. J. Dimler, B. E. Fisher and C. E. Rist, *Abstracts Papers Am. Chem. Soc.*, 124, 4D (1953).

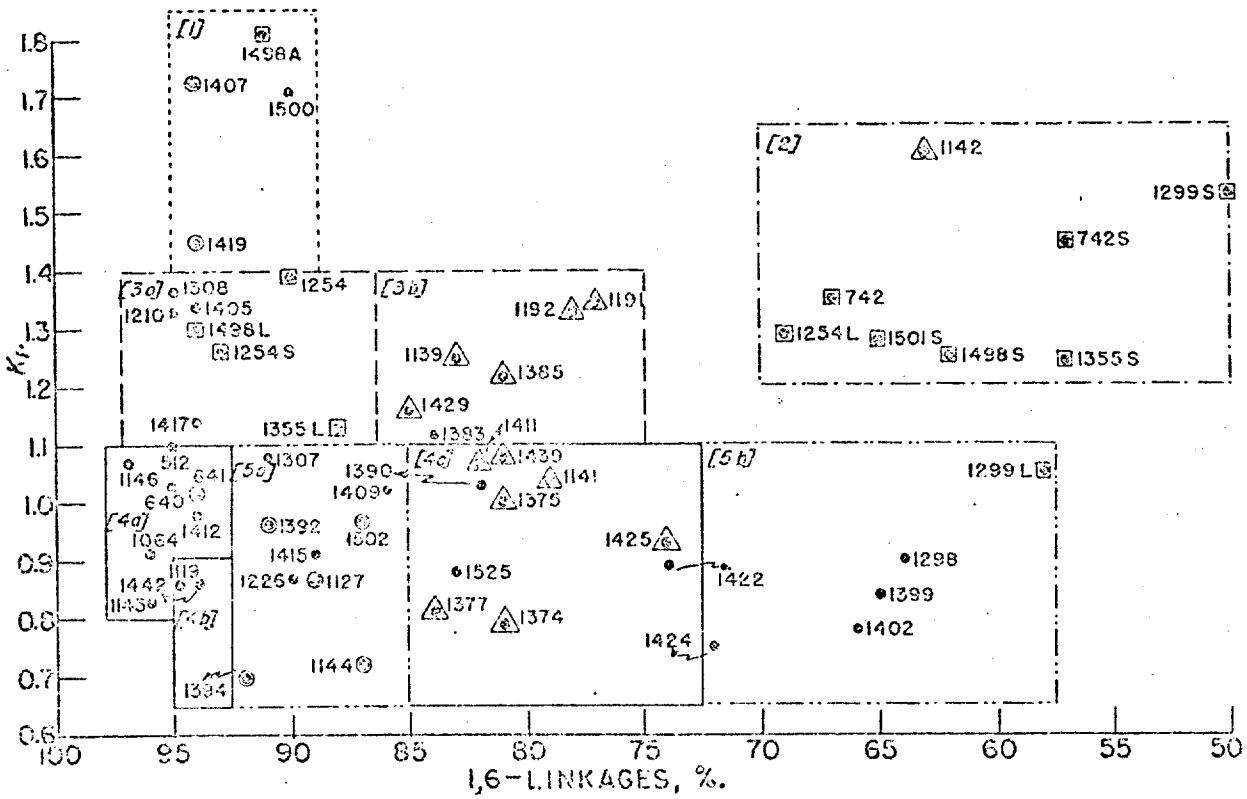

Fig. 2.—Relationships among k_1 parameter of water-soluble dextrans, per cent. of 1,6-links, and nature of the dextran precipitates.[a] The classification of the dextrans is indicated by: ⊛, A; ⊙, B; ⧋, C; ⊡, heterogeneous dextran group. The key to the group designations is given in Table IV.

[a] For a few dextrans, the properties of the precipitate agreed better with those of an adjacent group than with those of the group where plotted. This is indicated by an arrow from the plotted point to the dextran number.

and B-1443 in 4c). However, we do not wish to imply the expectation that the group demarcations in Fig. 2 will hold rigidly for all dextrans. Even for our own different preparations of dextran from strains B-512, -742 and -1146, respectively, the k_1 values showed a variation of about ±0.10.

Relationships between the Strains and Dextran Properties. Constancy and Reproducibility of Dextran Properties.—We have found that for many dextrans apparently exact reproduction of results was obtained in repeated preparations and that, for many of the strains, modifications such as those indicated in the Experimental section could be made in culturing conditions without detectable changes resulting in the dextrans.[24] Under other conditions, dextrans from a few strains have shown definite variations which might have resulted from more critical modifications in culture conditions or from changes in the culture itself or from differences in fractionation during purification of the dextrans. Thus, our first preparations of B-523 and of B-742 dextrans gave evidence of little or no 1,3-like links,[16] in contrast to the much higher contents of the corresponding preparations reported here.

The structure of our first preparation of B-742 dextran was in excellent agreement with that indicated by methylation-structure analysis on dex-

TABLE IV

CORRELATION OF DEXTRAN PROPERTIES WITH DATA SHOWN IN FIG. 2

Group	Class of dextrans[a]	Nature of dextran precipitate[b]	[η] (water, 25°)	Appearance of dilute aqueous soln.[c]
1	A, B	V. cohesive, tough, short gum	1.16–0.57	V. turbid
2	A, C	Fine or floc. ppt.	0.47–0.19	Marked bluish opalescence
3a	A, B	Fine or floc. ppt. to crumbly or dense short gum	0.89–0.47	V. turbid
3b	C	Floc. ppt. to crumbly or dense short gum	1.36–0.59	V. turbid
4a	A, B	Long, soft gum	1.28–0.95	Sl. opalescence
4b	A, B	Uniquely cohesive, stringy gum	2.00–1.62	Sl. opalescence
4c	A, C	Long, stringy or fluid gums	1.36–0.42	Clear to sl. turbid
5a	A, B	Short or stringy gums	1.20–0.56	Turbid or sl. opalescence
5b	A, B	Floc. ppt. or short gum	1.03–0.87	Sl. to v. turbid

[a] Dextrans or their components from the heterogeneous group are included in their respective classes. [b] Observed when precipitated from aqueous solution by ethanol of 45–60% concentration. [c] Approximately 1–2% concentration.

tran from this same strain.[16,52] Our subsequent preparations from this strain have contained 2 distinct structural types of dextran, one of which (fraction L or L-R) apparently was identical with previous preparations and the other (fraction C or S-R)[25] was an entirely new type having a high content of 1,3-like links. Characterization of dextran products from 12 different colonies picked from a plated culture of B-742 failed to reveal evidence of variation or mutation in the culture. These products had somewhat different proportions of the 2 structural types, 1,6-links from 72–75% and intrinsic viscosities near 0.20. The preparation reported in Table I (heterogeneous group), which was obtained from a large-scale fermentation of the original culture, had a higher content of the anomalous fraction and, therefore, showed lower 1,6- and higher 1,3-like linkage contents and higher viscosity and rotation.

The strains B-742 and B-1142 (class C) had a common origin but different subsequent histories. Our B-1142 dextran consisted almost exclusively of the anomalous fraction. Apparently these cultures have been changing, B-1142 more than B-742. A similar change in another strain is indicated by published data[9,53] and by our results on dextran from this strain (B-1375, Table I, class C).

Bacterial Classification and Dextran Type.—The fact that methylation analysis gave no evidence of branching in dextrans from 2 strains of *Leuconostoc dextranicum*[7,8,13] has led to the apparent expectation that all such strains would produce essentially a straight-chain type of dextran.[54] Our observations indicate that although several of our strains of *L. dextranicum* produced dextrans with low percentages of non-1,6-links and long gums (B-640, -1145 and -1146), others produced dextrans with high percentages of non-1,6-links and short gums (B-1120, -1141 and -1375). Dextran B-1193 had 95% 1,6-links, but short gum.

Acknowledgment.—We should like to express our gratitude to the numerous individuals and organizations named in Table I who gave us cultures utilized in this investigation. We are deeply indebted to Dr. E. J. Hehre for advice and encouragement and to members of this Laboratory, as follows: Dr. R. T. Milner for coördination of this project; C. H. vanEtten, T. A. McGuire and Mary Weile for the nitrogen, phosphorus and ash analyses; and Lenora J. Rhodes and Geraldine Bryant for assisting with production of the cultures and of the media, respectively.

(52) Published information[13] was the basis for our identifying strain NRRL B-742[16] ("number 5" of Hucker[17] and "culture 4" of Tarr and Hibbert[4]) with the strain whose dextran was subjected to methylation-structure analysis by Levi, *et al.*[14] Another source (Ph.D. thesis of I. Levi, McGill University, 1942) now has disclosed that this methylation study was made on dextran from either "culture 1" or "culture 2."[4] Fowler, *et al.*,[5] and later T. H. Evans (Ph.D. thesis, McGill University, 1941), carried out methylation-structure analysis on dextran from "culture 4" but obtained results almost identical with those obtained by Levi, *et al.*, on the other dextran.[14]

(53) S. A. Barker, E. J. Bourne, G. T. Bruce and M. Stacey, *Chemistry and Industry*, 1156 (1952).

(54) S. A. Barker and E. J. Bourne, *Quart. Revs.* (*London*), **7**, 58 (1953).

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363. FIBROSARCOMAS INDUCED BY MULTIPLE SUBCUTANEOUS INJECTIONS OF CARBOXYMETHYLCELLULOSE (CMC), POLYVINYLPYRROLIDONE (PVP), AND POLYOXYETHYLENE SORBITAN MONOSTEARATE (TWEEN 60). Norman M. Lusky and Arthur A. Nelson (intr. by Harold C. Hodge). Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

Groups of rats each received 1 cc weekly subcutaneous injections of one of the following aqueous solutions: CMC, 2%; PVP, 6%; Tween 60, 6%; dextran 6%; or sodium chloride, 0.9%. Each group of 30 rats was made up of 20 Osborne-Mendel evenly divided by sex, and 10 Bethesda Black males. After 73 wk the total number of rats with injection site tumors was CMC, 13 or 43%; PVP, 13 or 43%; Tween 60, 5 or 17%; dextran, none; sodium chloride, none. By rat strain and sex, the total number of injection site tumors was Osborne-Mendel male, 9; Osborne-Mendel female, 6; Bethesda black male, 16. No influence of the injections on spontaneous-type tumor production was seen, except that 1 rat on CMC and 3 on Tween 60 (these 4 did not have injection site tumors) had large intra-abdominal lymphosarcomas (the latter figure is higher than would be expected). Injection of CMC left persisting deposits of it at the injection site; this was not evident with the other materials. Microscopically, the injection site tumors were fibrosarcomas of moderate histological malignancy. Grossly, they ranged in size from 1.5 cm spherical to 10 x 6.5 x 4 cm. Hueper (Proc. Am. A. Cancer Res., 2:120, 1956) has reported PVP to be cancerigenic in rats.

Stability of Dextran during Prolonged Storage

So far as we are aware, no measurements relating to the stability of clinical dextran solutions kept for several years have been published. Two dextran solutions made in the United States, *A* and *B*, and two made in Great Britain, *C* and *D*, were examined in 1954, and again after storage for 5 yr. at 4° C. Where applicable the methods of testing described in the British Pharmacopœia[1] were used. The optical rotation of each dextran was determined and used in calculating the concentrations of solutions for the viscosity measurements (Table 1). Dextran *D* had undergone the most change; the changes in the others were relatively small.

The renal excretion of these dextrans was measured in rabbits (Table 2). The greater part of the dextran was excreted on the first day, less than 3 per cent on the second and less than 1 per cent on the third day. Two, three or four rabbits were used for each determination and the daily results averaged; Table 2 records the totals for 3 days after injection. The results for dextran *A* suggest a change in molecular composition, but no corroboration of this was found in the other measurements.

Table 1

Dextran	Optical rotation (*a*)	Intrinsic viscosity at 25° C. 1954	1959
A	195·1	0·249	0·248
B	197·7	0·254	0·251
C	201·2	0·394	0·388
D	198·1	0·362	0·326

Table 2

Dextran	Percentages of injected dose excreted 1954	1959
A	37·2	47·6
B	43·1	39·1
C	13·5	14·6
D	15·9	15·4

The retention of each dextran in the plasma was determined by taking the average of daily estimations in the same groups of rabbits (Table 3). During five years of storage little if any change had occurred in the dextrans affecting their retention in the circulation.

Table 3. Plasma Concentration of Dextran as Percentage of Concentration 10 min. after Injection

Dextran		10 min.	Day 1	2	3	4	5	6	7
A	1954	100	28·8	11·7	0·4	0·0	0·0	0·0	0·0
	1959	100	29·6	15·2	2·25	0·8	0·0	0·0	0·0
B	1954	100	29·2	13·8	3·6	0·0	0·0	0·0	0·0
	1959	100	30·0	14·1	2·0	0·6	—	—	0·0
C	1954	100	56·8	34·8	12·3	3·8	0·7	—	0·0
	1959	100	61·0	40·9	18·3	10·0	—	—	0·0
D	1954	100	60·5	40·2	22·8	12·8	5·1	—	0·0
	1959	100	56·5	38·7	22·0	—	—	3·3	1·2

The difference in molecular composition of American and British dextrans is well illustrated by these results. The lower average molecular weight of the American dextrans is associated with shorter retention in the circulation and greater renal excretion. The British dextran, of higher average molecular weight, would induce greater aggregation of red cells *in vitro*[2] and possibly *in vivo*, too.

From our observations we conclude that during the 5-yr. period there was little, if any, change in the molecular composition of these dextran solutions and none that would be noticeable in clinical use. It is probable that any limitation of the 'storage life' of dextran solution will be imposed by defects in the container and its closure rather than by any instability of dextran solution itself.

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[1] *British Pharmacopœia* (1958).

[2] Hardwicke, J., Ricketts, C. R., and Squire, J. A., *Nature*, 166, [illegible] (1950).

Nature 215(5099): 415-416. 1967.

Table 1. HYPERGLYCAEMIC AND GLYCOGENIC EFFECTS OF DEXTRAN AND 'SEPHADEX' DERIVATIVES IN FASTED RATS

Material administered	Blood glucose (mg/100 ml.)	Liver glycogen (mg/g)
Distilled water	85 ± 2·7	1·0 ± 0·33
Glucose	104 ± 2·7	6·8 ± 0·61
Dextran 60-*C**	101 ± 1·8	6·2 ± 1·47
DEAE dextran†	91 ± 6·3	5·6 ± 1·46
'Sephadex *G*-25'	87 ± 3·6	4·7 ± 1·97
DEAE 'Sephadex *A*-25'	81 ± 4·6	0·6 ± 0·37

Glucose was determined in whole blood by the potassium ferricyanide–potassium ferrocyanide method of Hoffman[6] adapted to the 'Technicon' auto-analyser. Although this method is sensitive to other blood reducing substances, normal concentrations of these substances are relatively insignificant compared with glucose levels. The results represent means ± standard error of the mean, ten animals in each group.

* Sigma Chemical Company, average molecular weight 75,000.
† Pharmacia Fine Chemicals, Inc., average molecular weight 2×10^6.

Table 2. HYDROLYSIS OF DEXTRAN AND 'SEPHADEX' DERIVATIVES BY HOMOGENATES OF SMALL INTESTINAL MUCOSA

Substrate	Hydrolytic activity (μmoles maltose equiv./60 min)
Dextran 60-*C*	8·66
DEAE dextran	2·44
'Sephadex *G*-25'	0·14
DEAE 'Sephadex *A*-25'	0·06

Twenty mg of substrate dissolved or suspended in 1 ml. of 0·1 molar maleate buffer (pH 6·0) and 1 ml. of homogenate were incubated for 60 min at 37° C. Hydrolytic activities were corrected for reducing substances present in the homogenate. The results are the means of duplicate samples.

Metabolism of Orally Administered Dextran and 'Sephadex' Derivatives in the Rat

DEAE 'Sephadex *A*-25' has oral hypocholesterolaemic activity in cockerels and dogs[1]. This material is thought to interrupt the enterohepatic circulation of bile acids by binding them in the intestinal lumen and enhancing their excretion in the faeces. DEAE 'Sephadex *A*-25' is a cross-linked tertiary amine derivative of dextran with an average molecular weight of 25,000 and is insoluble in water and salt solutions. As such it would not be expected to be absorbed intact from the gastrointestinal tract. The possibility that the polymer might be hydrolysed by intestinal enzymes and the hydrolysis products absorbed has not, however, heretofore been investigated.

Chemical methods are not readily available for detecting hydrolysis products of DEAE 'Sephadex', or of the intact polymer, in biological fluids or tissues. It has, however, been shown that oral administration of dextran leads to an early increase in blood sugar and liver glycogen in rats and man[2]. This is presumably the result of hydrolysis of dextran to glucose by intestinal enzymes[3]. To determine whether or not DEAE 'Sephadex' is metabolized in a similar manner, the hyperglycaemic and glycogenic effects of dextran and DEAE 'Sephadex', together with other dextran derivatives, were compared in fasting rats. The extent of hydrolysis of these materials by homogenates of small intestinal mucosa was also determined.

Male Sprague-Dawley rats, 243–325 g, which had been maintained on a semisynthetic diet, were fasted for 24 h. Animals then received by stomach tube 500 mg of the material to be tested, dissolved or suspended in 5 ml. of distilled water. 4 h later the animals were anaesthetized with an intraperitoneal injection of 'Cyclopal' [5-allyl-5(2-*cyclo*penten-1-yl)barbituric acid] and blood samples were taken from the abdominal aorta in heparinized syringes for analysis of glucose. Livers were excised, rinsed in saline, blotted dry and weighed. Samples of about 500 mg were dropped immediately into glass centrifuge tubes containing 1 ml. of 30 per cent potassium hydroxide. The tubes were heated in boiling water for 30 min. 3 ml. of distilled water and 5·5 ml. of 95 per cent ethanol were added and the tubes were placed again in boiling water to precipitate the glycogen. The tubes were cooled in tap water for 10 min and centrifuged. Supernatant fluid was decanted and the glycogen was dissolved in distilled water. Glycogen was determined by the anthrone method of Seifter *et al.*[4] using a glucose standard.

Intestinal mucosa homogenates were prepared from rats fasted for 24 h. The animals were killed by a blow on the head and the proximal two-thirds of the small intestine was excised, rinsed in cold saline and slit longitudinally on an iced glass plate. Mucosa was scraped off with the edge of a microscope slide and homogenized in 4 ml. of cold saline per g tissue in a 'Lourdes' tissue homogenizer at full speed for 1 min. The homogenate was centrifuged for 10 min at 2,000*g* at 0° C. The supernatant fraction was used to assay for hydrolytic activity according to the procedures of Dahlqvist[3,5] for determining amylase and dextranase activity. Hydrolytic activity is expressed as the increase in reducing power equivalent to 1 μmole of maltose for 60 min at 37° C.

Blood sugar concentration in rats dosed with glucose and dextran was significantly increased after 4 h (Table 1). In animals dosed with DEAE dextran, 'Sephadex *G*-25', or DEAE 'Sephadex *A*-25' blood sugar was not significantly different from controls given distilled water. Liver glycogen increased between five and seven times in rats given glucose, dextran, DEAE dextran, or 'Sephadex *G*-25'. DEAE 'Sephadex *A*-25', on the other hand, produced no noticeable change in liver glycogen. An apparent discrepancy exists because DEAE dextran and 'Sephadex *G*-25' were markedly glycogenic but did not increase blood sugar. On the other hand, in a previous study[2] maximum elevation of blood sugar occurred in rats 0·5 to 2 h after dosing with 450 mg of dextran, and blood sugar concentration had returned to control values after 4 h. It is therefore probable that blood sugar was elevated by DEAE dextran and 'Sephadex *G*-25' also, but that this was not detected when samples were taken after 4 h.

The results in Table 2 show that DEAE dextran is extensively hydrolysed *in vitro* by an enzyme preparation previously shown to hydrolyse dextran itself[3]. 'Sephadex *G*-25', an insoluble cross-linked dextran, is also hydrolysed, although to a much smaller extent, while DEAE 'Sephadex *A*-25' is not affected significantly.

These experiments indicate that DEAE 'Sephadex *A*-25' is resistant to degradation in the intestinal lumen. It seems unlikely therefore that any appreciable amount

of this material could be absorbed after oral administration.

I thank Mrs F. L. Schmidt for performing the blood glucose analyses and J. C. Schneider, jun., for his expert technical assistance.

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INDUCTION OF SARCOMA IN THE RAT BY IRON-DEXTRAN COMPLEX

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[WITH SPECIAL PLATE]

Pathological amounts of iron may accumulate in the body in a variety of conditions—for example, haemochromatosis and transfusional, malnutritional, and occupational siderosis. In all these conditions arguments for and against the cytotoxic effects of iron have been advanced, but the possibility that this metal plays any part in carcinogenesis has hardly been entertained. Therefore, in the belief that iron was non-carcinogenic if not altogether bland, an iron-dextran complex ("imferon," Benger Laboratories Ltd.) was deliberately chosen in order to establish a progressive mobilization or "hyperplasia" of histiocytes. This need arose when it had been discovered (Richmond, unpublished observation) that repeated subcutaneous injections of trypan blue commonly induced a pleomorphic histiocytic sarcoma in the rat at the site of injection. By a collateral series of experiments with a non-carcinogenic substance it was hoped to detect some critical difference between a "hyperplasia" and a neoplasia of histiocytes. This hope was not realized because another crop of pleomorphic histiocytic sarcomas histologically identical with those induced by trypan blue was obtained as detailed below.

All the rats belonged to a home-bred hooded strain in which no spontaneous tumours of this kind have previously been recognized in this laboratory. They were fed on Diet No. 1 (Thomson cube) supplemented with greens.

Experimental Procedures

Experiment 1.—The animals were all adult males within the weight range of 250–300 g. at the beginning of the experiment. (*a*) 40 received a weekly intramuscular injection of 0.4 ml. iron-dextran complex into the right upper thigh. Each dose contained 20 mg. of iron as ferric hydroxide in complex with low-molecular-weight dextran. (*b*) 12 received weekly intramuscular injections of 0.5 ml. "ferrivenin" (Benger) under ether anaesthesia, with occasional interruptions owing to the development of ulceration. Each dose contained 10 mg. of iron as saccharated oxide of iron. (*c*) 12 received weekly injections of 0.5 ml. low-molecular-weight dextran. (*d*) 12 received weekly injections of 0.5 ml. normal saline solution.

Experiment 2.—The animals were weanling rats of both sexes averaging 48 g. in weight. (*a*) 20 males and 20 females were given twice-weekly intramuscular injections of iron-dextran complex into the right upper thigh in graduated dosage according to weight—namely, 0.1 ml. up to 100 g., 0.2 ml. up to 150 g., 0.3 ml. up to 200 g., and 0.4 ml. thereafter. This regimen was stopped after three months, each animal

having received 9.5 ml. iron–dextran complex. (*b*) 6 males and 6 females received corresponding volumes of low-molecular-weight dextran solution.

Results

In Experiment 1, 17 of the 40 animals receiving iron-dextran complex were killed during the early months, but 16 of the remaining 23 developed tumours at the site of injection between the 11th and 16th months of the experiment. Rats given low-molecular-weight dextran and saline did not develop tumours, nor did those animals receiving saccharated oxide of iron over a period of 17 months. In the last group the injections commonly gave rise to necrosis which might be associated with ulceration of the overlying skin and secondary infection; collections of siderocytes and an abundant granulation-tissue reaction were demonstrated on histological examination, but no evidence of malignancy was seen.

In Experiment 2, tumours developed in 10 female and 12 male rats at the site of injection from six to eight months after cessation of iron treatment, while those receiving dextran alone showed no tumour formation.

Histological Observations.—The essential change at the site of injection of the iron-dextran complex in the early stages is a progressive accumulation of histiocytes laden with iron pigment. Previous to the inception of tumours it has been established from biopsy material that occasional histiocytes develop enlargement and hyperchromatism of the nucleus associated with mitotic activity and other aberrant changes (Special Plate, Fig. 1). Therefore it is deduced that the neoplastic process originates in such histiocytes, which gradually lose their avidity for iron as the neoplasia gathers momentum. The characteristics of established tumours vary from spindle-cell sarcoma to a highly pleomorphic growth including many giant cells and exhibiting numerous mitoses (Special Plate, Fig. 2). Iron-laden histiocytes are distributed throughout the tumour, and, while some neoplastic cells contain traces of the metal, the great majority are free from it.

Transplantation.—Metastases were not observed in the tumour-bearing animals, but transplantation of the tumour was successful in three of four examples in which this was attempted. In the first generation the transplants grew slowly after a lag period of four, six, and eight months respectively, but more rapidly in subsequent generations. One line is now in the 27th generation, requiring transplantation every four weeks. Histologically, some degree of the initial pleomorphism is still seen in this transplanted tumour, but the growth is essentially a spindle-cell sarcoma.

Discussion

From these observations it is clear that intramuscular injection of iron–dextran complex is carcinogenic in the rat. It is also clear that the dextran fraction alone is free from carcinogenic activity under the conditions of the experiments, so that iron is likely to be the responsible agent. The possibility that this effect is exerted only when it is in complex with dextran has been considered, but this is regarded as unlikely owing to the fact that the dextran is rapidly split off after injection and the iron chelated with protein to form haemosiderin (Golberg, 1957). The attempt to decide the issue by the repeated injection of saccharated iron was upset by the occurrence of necrosis and ulceration, necessitating interruptions in the sequence of the injections. These findings in the rat following the injection of iron–dextran complex have been confirmed in a small series of mice at the Chester Beatty Research Institute (Haddow, 1958).

The clinical significance of these observations cannot be assessed pending an elaborate study of the many factors involved. The iron–dextran complex has been widely used in the treatment of iron-deficiency anaemias in adults (Scott and Govan, 1954; Cappell *et al.*, 1954; Baird and Podmore, 1954; Jennison and Ellis, 1954; Scott, 1956) and in infants (Gaisford and Jennison, 1955). The amount administered in the adult human subject will vary according to the need of the patient, up to 54 ml. being recommended by the manufacturer for a patient weighing 180 lb. (81.64 kg.) with an observed haemoglobin value of 50%. Against this may be contrasted the volume administered to the rat—namely, 17.6–25.6 ml. in Experiment 1 (*a*) and 9.5 ml. in the younger rats of Experiment 2 (*a*). Therefore it will be evident that the dosage used in these experiments is relatively massive—some 200–300-fold—when compared with the therapeutic dose—for example, 20–40 ml. for a 70-kg. man.

Hitherto iron has not been proved to be a carcinogenic agent, and this finding may have some wider implications. For example, frequent reports appear in the literature concerning the high incidence of hepatoma supervening on the cirrhosis of the liver which is associated with haemochromatosis. The usual incidence of liver cancer in relation to cirrhosis of Laennec type has been placed at 3 to 7% (Anderson, 1957). In contrast, Warren and Drake (1951) reported the development of hepatoma in 18% of their cases of haemochromatosis, Stewart (1931) found an incidence of 10% in 151 collected necropsy cases of haemochromatosis, and Willis (1953) found three cases of hepatoma in seven necropsies on patients suffering from haemochromatosis. These figures suggest that there is a special tendency for the cirrhosis of haemochromatosis to undergo malignant change as compared with cirrhosis in general. While Edmondson and Steiner (1954) believed that the difference might be due to patients with haemochromatosis living longer than patients with portal cirrhosis of Laennec type, the present experiments suggest that gross iron deposition may well be the factor responsible. Incidentally, Branwood (1958) has investigated 100 examples of portal cirrhosis, of which slight to gross deposition of haemosiderin in the liver cells was observed in 33 cases. Hepatoma supervened in six of the 100 cases of portal cirrhosis, and all six were included in the group showing haemosiderosis.

The evidence submitted also suggests that iron may be the important causal agent in the development of pulmonary carcinoma in haematite miners. The rising incidence of pulmonary carcinoma in haematite miners was pointed out by Faulds and Stewart (1956), who found 17 cases of carcinoma of the lung in 180 necropsies (9.4%). These lungs contained silica as well as iron, but there was no similar incidence of pulmonary carcinoma in coal-miners dying of silicosis in the same area of West Cumberland, nor had this been reported from other coal-mining areas. Faulds added more information in 1957 with regard to the incidence of pulmonary carcinoma in haematite miners, making it clear that the only other silicious trades associated with

an increased incidence of carcinoma of the lung were asbestos workers, moulders and foundrymen, and chromate workers, and pointing out that asbestos and chromate contain varying amounts of iron in their chemical composition. In this context there is further support from the work of Campbell (1940, 1943), who exposed mice of a strain susceptible to lung cancer to an atmosphere laden with ferric oxide. He found that 32.7% of the mice exposed to the dust developed tumours of the lung compared with 9.6% of the controls, while mice exposed to a mixture of silica and ferric oxide showed a tumour incidence halfway between these figures (19.4%).

Mode of Action of Iron.—It has been observed that the histiocytes at the site of injection and elsewhere in the body (for example, Kupffer cells) contain, in addition to iron pigment, globules of a lipofuscin pigment of ceroid nature. The development of ceroid throughout the tissues of the body is one of the characteristics of vitamin-E deficiency (Mason, 1944), and in recent experiments Golberg and Smith (1958) have shown that the ceroid developing through iron overload can be largely prevented by supplementing the diet with α-tocopherol. It may therefore be inferred that one way in which iron influences intracellular metabolism is by blocking the antioxidant activity of vitamin E and possibly other antioxidants in the cell. The destruction or interference with these natural antioxidants allows oxidation of unsaturated fats to form the yellow pigment which is termed ceroid (Casselman, 1951). It remains to be shown whether this action of iron in the cell has any relation to the development of malignant change.

Summary

In the adult rat weekly-repeated intramuscular injections of iron–dextran complex induced sarcoma at the site of injection. A bi-weekly series of similar injections, begun in weanling rats and stopped after 12 weeks, also induced sarcoma at the site of injection some seven months later.

I wish to thank Professor J. S. Young for continuous encouragement and advice during this work, and Professor A. Haddow for a helpful discussion and the personal communication of his observations on the mouse. My thanks are also due to Mr. A. Bodie, senior technician, for his co-operation, and to Miss E. M. Gillies, who carried out much of the technical work. Mr. W. Topp kindly supplied the photographs.

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Biosynthesis of the C^{14}-Labeled Form of Dextran[1]

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Dextran is a polysaccharide, made up solely of glucose units, produced by bacterial fermentation of sucrose. Only the glucose portion of the sucrose molecule is utilized in the biosynthetic process. Dextran of suitable molecular size and purity is currently of interest as a synthetic plasma volume expander, particularly for use in event of large-scale catastrophe in which natural blood plasma supplies might be limited. Dextran has been tested clinically with success, but its metabolic fate in the body is inadequately known, since the best analytical procedures account for only about half of injected dextran. It was concluded that these metabolic questions could best be resolved through the use of an isotopically labeled form of dextran.[4]

Argonne National Laboratory and Commercial Solvents Corporation, at the request of the Office of the Surgeon General of the U. S. Army, and under the direction of the Subcommittee on Shock, National Research Council, cooperated in biosynthesizing C^{14}-labeled dextran at two different levels of activity, one designed for use in animal experiments, the other for human clinical experiments. Since 1949 the Research and Development Laboratories, Commercial Solvents Corporation, have conducted a dextran research program and at present have developed a successful clinical grade of dextran. The present report outlines the experimental procedures and results incident to the successful completion of the biosynthesis program.

Essentially, the problem involved the biosynthesis of labeled C^{14} sucrose, followed by the biosynthesis of labeled dextran through fermentation of this sugar. The quantity and absolute activity of labeled dextran required for proposed laboratory and clinical experiments necessitated the handling of approximately 0.3 c of radiocarbon. In order to determine the adequacy of both proposed experimental equipment and procedures, a small quantity of low absolute activity C^{14} dextran was biosynthesized. As soon as these studies were evaluated large-scale biosyntheses were initiated.

C^{14} sucrose was biosynthesized by allowing carbohydrate-depleted, excised *Canna* leaves to photosynthesize in the presence of $C^{14}O_2$ in a 38-liter hermetically sealed, leaf-chamber system.[5] This plant was selected because of its reported high efficiency in converting $C^{14}O_2$ to sucrose during photosynthesis (*1*). A total of 309.7 mc of $C^{14}O_2$ was generated from $BaC^{14}O_3$ in eight experiments employing a total of 30 leaves, weighing 208.8 g fresh. The separate photosynthesis periods varied from 6 to 24 hr and resulted in fixation of 308.0 mc in the leaves, or 99.4% of that generated.

A total of 175.2 mc of C^{14} sucrose was biosynthe-

FIG. 1. Radioautograph of paper chromatogram of five separate lots of C^{14} sucrose showing their lack of contamination. Paper partition chromatogrammed for 48 hr at 20° C with BuOH : EtOH : H_2O 45 : 5 : 50 as irrigating solvent. Radioautographed for 30 days using Eastman No-screen x-ray film.

[1] The experimental work was carried out in the Division of Biological and Medical Research, Argonne National Laboratory, under Contract No. DA-49-007-MD-102 between Commercial Solvents Corporation and the Office of the Surgeon General of the U. S. Army. The authors are greatly indebted to Weldon Brown, University of Chicago, and to F. H. Schultz, Jr., Commercial Solvents Corporation, for their interest, encouragement, and counsel. They also acknowledge the able assistance of Lt. Col. E. J. Pulaski, Army Medical Center; Phillip H. Abelson, Carnegie Institution; Walter L. Bloom, Emory University, and A. M. Brues, Argonne National Laboratory, in planning various phases of this project.

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[4] The metabolism studies are being conducted by various investigators, at the direction of the Subcommittee on Shock, National Research Council.

[5] The detailed experimental methods utilized for the biosynthesis and isolation of C^{14} sucrose and other plant fractions from Canna leaves are to be presented elsewhere.

sized. A total of 741.8 g of pure carrier sucrose was added to the isolated carbohydrate leaf extracts, utilizing the isotope dilution technique, and 681.9 g of crystalline sucrose containing 168.4 mc was obtained. The average activity was 245.9 μc/g of sucrose, and the average sucrose extraction efficiency was 96.1%. On the basis of the total quantity of C^{14} fixed in photosynthesis, the over-all efficiency in conversion into sucrose was 56.9%. With certain individual leaf biosynthesis runs, the sucrose efficiency was as high as 72.1%. Fig. 1 shows a radioautograph of a paper chromatogram of five separate lots of crystalline C^{14} sucrose and indicates the lack of contamination. Two of the five lots of sucrose were degraded, using enzymatic and microbiological methods (*2*), and were found to be uniformly labeled.*

Identical fermentation techniques were employed for the biosynthesis of dextran at each of the two different activity levels—namely, 106.8 μc/g for animal experimentation and 5.68 μc/g for use in human beings. The bacterium *Leuconostoc mesenteroides* was employed in these fermentations. The medium used was that described by Jeanes, Wilham, and Miers (*3*). Fermentations were conducted in 1-, 4-, or 6-liter flasks, containing 300, 3000, or 4000 ml of medium, respectively, sterilized at 116° C for 30 min. Small inoculum cultures used for seeding large-scale C^{14} fermentation flasks were prepared with C^{14}-labeled sucrose, and the entire contents of the culture flask were added equally to each fermentation flask. The fermentation period was terminated during the 26th hour.

Because of the large volumes involved, a total of seven fermentation runs was necessary, five for the low activity level dextran and two for the high activity level material. The native dextran was precipitated from the fermentation beer with methanol. The crude dextran was dissolved in water and reprecipitated with methanol. This was repeated several times more for purification.

For depolymerization the native dextran was subjected to acid hydrolysis, the course of which was followed by determination of viscosity change. The hydrolysates were cooled rapidly, made slightly alkaline with sodium hydroxide, and filtered through a layer of filter aid. The hydrolyzed dextran was fractionated and refractionated from aqueous solution by the addition of methanol, separating the material into three fractions: high molecular weight, intermediate (clinical), and low molecular weight. Wherever necessary the determination of dextran in solution was carried out by the anthrone method (*4*).

In the preparation of the dextran for animal experimentation 303 g of C^{14} sucrose having an absolute activity of 114.7 μc/g was fermented. The clinical fractions from two hydrolyses were reprecipitated together from aqueous solution by the addition of methanol. The dextran was dried, ground to 40 mesh in a Wiley mill and packaged in 1.0 g units. The yield of dry, pyrogen-free dextran of clinical size was 25.3 g containing 2.70 mc of C^{14}. The specifications of the final product are listed in Table 1.

TABLE 1

SPECIFICATIONS OF C^{14} DEXTRAN FOR ANIMAL AND CLINICAL EXPERIMENTS

Characteristic	Dried dextran for animal use	Dextran in solution for human use
Sterility to yeasts, molds, and bacteria	—	Passed
Average dextran concentration, g/100 ml	—	6.06
Viscosity of a 6% solution at 25° C (centistokes)	3.25	2.79
Intrinsic viscosity at 25° C	—	0.20
Mol weight (wt av by light scattering)	126,000*	82,600*
Nitrogen, mg/100 ml	—	0.56
pH	—	5.55
μc/g on solid	106.8†	5.68‡
μc/g based on carbon recovered in combustion	116.8†	6.35‡

* Determined by E. E. Toops, Jr., Commercial Solvents Corporation.
† Assayed by Alexander Van Dyken, Chemistry Division, Argonne National Laboratory.
‡ Assayed by K. E. Wilzbach, Chemistry Division, Argonne National Laboratory.

For the preparation of dextran for human experiments, purified native dextran obtained from the fermentation of 17,800 g of C^{14} sucrose (5.99 μc/g) was hydrolyzed in two batches. Hydrolyses were carried out in twin circular white-enameled laundry tubs, insulated externally, and fitted with glass coils for steam heating and water cooling. All exposed metallic surfaces were coated with Tygon plastic, and the tubs were covered during the hydrolysis with Tygon plastic sheeting. Hydrolysates were pumped through Tygon tubing by means of a glass-lined centrifugal pump into a ceramic filter (diameter, 3 ft) and filtered by suction through a layer of filter aid. Fractionation was carried out as noted above. The final sterile and pyrogen-free product was made up to a 6% dextran solution to which 0.9% sodium chloride had been added. It was bottled in 500-ml units. A total yield of 68 bottles containing 12.58 mc was obtained. The specifications of the final product are listed in Table 1.

Radioactive dextran has been placed in the hands of a number of investigators.† Preliminary results definitely indicate that dextran is metabolized by mice, rats, dogs, and human beings, with a substantial portion of the labeled dextran appearing in the expired air as $C^{14}O_2$. One preliminary report has already

* The authors wish to thank Martin Gibbs, Brookhaven National Laboratory, Upton, Long Island, N. Y., for conducting the degradation studies.

† J. Garrott Allen, University of Chicago Medical School; Walter L. Bloom, Emory University Medical School; Leon Hellman, Sloan-Kettering Institute; Joe Howland and Rodger Terry, School of Medicine and Dentistry, University of Rochester; Surgical Research Unit, Brooke Army Hospital, Fort Sam Houston, Texas; and Harry M. Vars, School of Medicine, University of Pennsylvania.

been published (*5*). Detailed publications will be forthcoming from the various investigators and will appear elsewhere.

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DEXTRAN

Its Properties and Use in Medicine

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CHAPTER I

PLASMA SUBSTITUTES

AN ideal plasma substitute should possess all the attributes of plasma itself. None of the substances which have been used, however, can be regarded as a plasma substitute in this sense. They all lack, among other things, antibodies, enzymes and clotting factors, possess only slight buffering power, and with the exception of gelatin, do not contain protein and therefore cannot perform the nutritive and carrier functions of the normal plasma proteins. They resemble plasma mainly in their ability to exert a colloid osmotic pressure and it is upon this similarity that a reasonably satisfactory definition can be based. The term plasma substitute is used for any substance, a solution of which, when infused intravenously, will restore a diminished blood volume, and maintain it for a longer or shorter time, in a manner similar to that of homologous plasma or serum.

The use of plasma substitutes arose from the physiological laboratory technique of the perfused isolated organ or limb, in which the nature of the perfusing fluid must be such that the tissues of the limb are in a condition as close to normal as possible. It was early realised that solutions containing only crystalloids were not suitable for such experiments on mammalian tissues, but not before many and complex types of solutions had been tried. Mammalian tissues perfused with crystalloid solutions soon became oedematous and abnormal. The significance of the blood colloids in keeping a normal water balance, was appreciated by Claude Bernard in the middle of the last century, and Karl Ludwig (1863) had observed that "urine" formation was maintained in a pig kidney perfused with 3 per cent gum arabic in 1 per cent sodium chloride. The classic experiments of Starling (1896), however, must be taken as the starting point of the modern conception of the importance of blood colloids in controlling the passage of fluid across the capillary membrane and in maintaining the blood volume. The colloid osmotic pressure exerted by the plasma proteins, of which albumin by virtue of its molecular size and concentration is the most active, tends to draw

fluid from the tissues through the capillary walls into the blood; this pressure is opposed by the blood pressure. The balance between these two opposing forces determines in the main whether fluid will enter or leave the circulation through the capillary walls.

The knowledge gained in the physiological laboratory was applied to the treatment of oligaemic "shock" in the Great War, 1914–1918. The importance of blood transfusion and the relatively large amounts of blood needed had not been appreciated before. Supplies of whole blood, the fluid of choice, were relatively limited, however, because methods of preserving blood were still undeveloped, and the highly organized transfusion services, which played so important a part in the Second World War and now known to be essential, did not exist. Two solutions containing colloids were used to supplement whole blood. In 1915 Hogan described the successful resuscitation of six patients by the infusion of 2.5 per cent gelatin in hypertonic salt solution, but gelatin solution was not widely used. Gum-saline solution, first tested experimentally by Bayliss (1916), who demonstrated experimentally its superiority over saline solution, rapidly won favour and in 1917 was successfully used for the treatment of oligaemic shock (Horwitz, 1917; Drummond & Taylor, 1919; Bayliss, 1919), and continued to be used for many years. Gradually evidence of harmful or undesirable effects accumulated; Studdiford (1937) described severe reactions, Andersch and Gibson (1934) and Dick *et al.* (1935) observed storage of gum in the liver and other organs, and Keith *et al.* (1935) reported that gum might persist in the blood stream for very long periods after infusion. In the Second World War interest in plasma substitutes was renewed, because it was feared that the supplies of blood and plasma provided even by the extensive transfusion services might be insufficient for the treatment of the large numbers of casualties expected. This fear proved to be unfounded.

The interest awakened during the war has become even more active and intense since the war ended, because safe and effective transfusion fluids are needed in those parts of the world where, for climatic, financial and other reasons, transfusion services cannot be organized, and because provision of enough blood and plasma would probably be beyond the capacity of any country in a future calamity involving modern weapons. Furthermore, in

an emergency of this kind it would probably be impossible to perform even a restricted range of blood grouping and compatibility tests, so that a plasma substitute would often be given in place of blood in transfusions for which, under normal circumstances, blood would have been used.

To be acceptable, a plasma substitute should possess certain desirable qualities, which may be conveniently regarded as positive and negative.

POSITIVE QUALITIES DESIRED

(1) It has been generally agreed that when dissolved to make a solution the substance should exert a colloid osmotic pressure of the same order as that of plasma. Dextran as generally supplied however has a colloid osmotic pressure somewhat greater than that of plasma. It has been generally assumed that a solution consisting of this dextran and serum in equal parts will have a colloid osmotic pressure equal to their mean. However, the osmotic behaviour of mixtures of dextran with serum albumin or with whole serum does not support this assumption (Wales *et al.*, 1954; Rowe, 1954) and it is suggested that the desirable colloid osmotic qualities of solutions of plasma substitutes would probably be more realistically defined in terms of the colloid osmotic pressures exerted by agreed mixtures of serum and plasma substitute. The reader is referred to pages 34 *et seq.*

For the same reasons, although it has long been agreed that the relative viscosity of a plasma substitute should be similar to that of blood or plasma, consideration should be given to redrawing this definition in terms of mixtures of serum and plasma substitute. *A priori*, it is desirable that the relative viscosity at 37°C of a plasma substitute or of mixtures of plasma substitute and serum should be similar to that of plasma.

(2) The substitute should be retained in the blood stream until its place has been taken by the normal plasma proteins. Most of the substances which have been used as substitutes are removed from the blood stream by the reticulo-endothelial system, by being completely or partly metabolised, by excretion through the kidneys or in the bile or digestive secretions, or by a combination of these methods. In practice retention in the blood stream is important if reliance has to be placed solely or largely upon the use of a plasma substitute and if blood and

plasma cannot be given at all or only in inadequate amounts; if, on the other hand, adequate supplies of blood and plasma are accessible but not immediately available, retention within the blood stream may not be of such great importance. For general purposes, however, any substitute should meet as nearly as possible the theoretical requirement mentioned, which implies that it should not be rapidly metabolised or otherwise removed and that only a small proportion should be excreted in the urine. Various criteria have been adopted with the object of meeting this requirement. In Great Britain for official procurement it is required that not more than 25 per cent should be excreted in the urine in the first 24 hours (Maycock, 1952). In the United States it has been recommended that a satisfactory substitute should maintain the plasma volume and keep a circulatory level such that 50 per cent of the amount infused is present for at least 12 hours, and preferably for 24 hours, after infusion (Richards, 1951).

(3) The molecular composition of a plasma substitute is of fundamental importance. All of the substances used are polydisperse and, although the degree of polydispersity can be diminished by fractionation, the fraction selected is always composed of molecules having a range of sizes about a mean. The proportion of small molecules which would pass rapidly through the renal glomeruli should be small; likewise the number of large molecules, which are associated with certain undesirable effects, for example, increased erythrocyte sedimentation rate, prolonged storage in the tissues, should also be small. The optimum means and distributions of molecular size of the various plasma substitutes have still to be determined.

(4) The composition of each batch should be constant within narrow and definable limits. The bulk production of substances of this type is complex, and variations between batches may occur unless their preparation is carefully controlled and standardized. It is clear that the molecular composition of a given substitute made in one country may differ, perhaps greatly, from that of one prepared in another country, depending upon the specification and method of preparation. Comparison of clinical and experimental results from different sources is impossible unless the plasma substitute is fully described. International agreement is desirable upon the optimum specification of each of the plasma

substitutes, and upon the physical, chemical and biological methods to be used for their characterization. No final criteria for clinical evaluation can be laid down, but with more experience, international understanding of the advantages of the various available methods should increase,

(5) The solution should be stable during storage without refrigeration.

(6) It should withstand sterilizing by autoclaving.

(7) It should be crystal clear so that contamination or other changes can be easily detected.

(8) It should be fluid at temperatures above 0°C.

NEGATIVE QUALITIES DESIRED

(1) The substance should not be antigenic.

(2) It should not be toxic locally or generally; for example, injection of the substitute should not cause thrombosis nor thrombo-phlebitis, nor tissue necrosis if it escapes from the vein, nor any general ill-effects such as fever.

(3) It should not cause permanent histological changes, nor should it remain in the tissues for long periods. Ideally, as already mentioned, the substitute should be completely eliminated from the body at a rate which will allow its persistence in the blood stream until its place has been taken by the normal proteins. In fact, some substitutes which have been used are eliminated so quickly that their effect is not much superior to that of saline, and others remain in the tissues for shorter or longer periods after they have disappeared from the blood stream. Histological changes have been described after the injection of many of the materials used as substitutes. Their significance is not always clear.

(4) It should not act as a diuretic and should cause no increase, or only a slight increase, in erythrocyte sedimentation rate. The latter effect can be reduced by eliminating the large molecules, but its abolition is impossible in most instances unless the mean molecular size is reduced to such an extent that the plasma substitute is very rapidly excreted.

[illegible] SUBSTITUTES

Some of the substances which have been used as plasma substitutes, present [illegible] mentioned. Some

approach more nearly the ideal than others. Dextran solution is one of these; it exhibits all the positive qualities, but lacks several of the negative qualities. Under certain circumstances dextran appears to act as an antigen in man (see p. 40) and, like all the substances hitherto used as plasma substitutes, it accelerates the erythrocyte sedimentation rate (see p. 28). It has also been shown to cause a defect in the haemostatic mechanism in normal individuals (see p. 53) and possibly also to enhance the infectivity of certain organisms in mice (see p. 54). On the other hand, dextran solutions of appropriate molecular composition have been administered with success for the resuscitation of large numbers of patients with oligaemic shock, and no evidence has yet been forthcoming that use of the solution has had any transitory or permanent harmful effects, apart from a small number of reactions probably of an allergic nature (see p. 67).

Dextran solution and plasma have one important property in common, that of exerting a colloid osmotic pressure. Both fluids are used clinically with approximately equal success with the main object of restoring a diminished blood volume to normal, but in assessing their relative merits consideration must also be given to the fact that, whereas plasma is a normal body fluid containing naturally occurring substances of various kinds and with many different properties, dextran solution contains only dextran and certain inorganic salts. Although plasma carries the risk of transmitting the infective agent responsible for homologous serum jaundice, the incidence of this complication of plasma has been greatly lessened in recent years by the introduction of "small pool" dried plasma, and in a recent survey (Ministry of Health, Medical Research Council and Department of Health for Scotland, 1954) was shown to be very low indeed (0.12 per cent) and not greater than that following the transfusion of whole blood (0.16 per cent). In places where adequate transfusion services exist, the provision of sufficient dried "small pool" plasma should present no technical difficulties.

On the other hand, as will be seen in subsequent chapters, the dosage of dextran needed to restore plasma volume in all but severe instances of diminished blood volume only involves some dilution, and not the complete replacement, of the normal protein constituents and formed elements of the blood, and this for only a period of hours. The extent to which a plasma substitute is

theory can only be finally determined by knowing how the body, perhaps already injured or diseased, will tolerate a lowering of concentration of the various normal constituents of the blood. The most critical of all these is probably the red cells, which are also the most slowly replaced constituent, so that for all but moderate haemorrhage, neither plasma nor plasma substitutes afford adequate therapy. It is not yet known which of the other constituents of blood can least be spared, but under many circumstances, no obvious bodily change results from their dilution for short periods to about half the normal level. Until more is known of the separate functions of the various plasma constituents, a conservative view may well be held, namely that dextran solutions and other plasma substitutes probably have their greatest part to play (1) in normal transfusion practice while waiting for compatibility tests to be done, and when plasma is not available, (2) in places where adequate transfusion services do not exist for climatic, geographical or financial reasons, and (3) in national emergencies. This statement of opinion perhaps anticipates the conclusions of subsequent chapters; in any case, the study of how dextran succeeds or fails as a plasma substitute is important, not only in relation to these useful roles, but in throwing light on the modes of action of foreign or natural macromolecules within the body.

Chapter II

CHEMISTRY OF DEXTRAN

NATURE AND SOURCE OF DEXTRAN

DEXTRAN is a collective name for a series of polyglucoses having a high dextrorotation of the order of +200° (specific rotation). Native dextran was first observed in sugar refineries as masses of slime which attracted attention because they impeded purification of the sugar. Pasteur in 1861 recognized that the slime was due to microbial action. Since then microbiologists have isolated various species of cocci belonging to the genus *Leuconostoc*, two of which, *L. mesenteroides* and *L. dextranicum* (Hucker and Pederson, 1930), under suitable conditions can produce dextran. As a rule, on solid media containing sugar, these organisms produce spreading mucoid colonies. (Another species, *L. citrovorum*, does not produce dextran.) Various strains of the same species of leuconostoc produce dextrans which differ slightly in chemical structure, and minor differences in the product can also be effected by variations in culture conditions. A review of various bacterial dextrans has been presented by Stacey and Ricketts (1951). As leuconostoc grows well on relatively simple fluid media, dextran can be readily produced on a very large scale by fermentation processes.

The primary requisite for dextran production by suitable strains of leuconostoc is the presence of sucrose. This is acted on by an exocellular enzyme which transfers the glucose moiety of sucrose to a suitable receptor or "starter" which may be another molecule of sucrose or the non-reducing end of a growing dextran chain. Certain other substances such as maltose and alpha-methyl glucoside can accept a glucosyl group and thereby initiate dextran formation. The early work in this field is due to Hehre (1941, 1946, 1948) and more recently investigations have been continued by the group at the Northern Regional Research Laboratory of the U.S. Department of Agriculture (Tsuchiya *et al.*, 1953)[1] Dextran formation has been compared with the formation of other polysaccharides in the review by Barker & Bourne (1953). Cell-

[1] From the same laboratory, Jeanes (1952), an active investigator in this field, has produced a valuable selected bibliography.

free enzyme preparations with high potency in converting sucrose to dextran have been obtained but the enzyme has not yet been crystallized (Koepsell & Tsuchiya, 1952). By analogy with the starches and glycogens where one enzyme is required for the formation of each type of linkage present, it seems likely that more than one enzyme will prove to be involved in dextran synthesis. Research on the fermentation process directed toward the kind of acceptors for glucosyl groups and upon the proportions of the various enzymes present is likely to lead to greater control over the kind of dextran produced and the length of the molecular chains. As will be apparent from later chapters these factors determine the suitability of dextran as a plasma substitute.

MANUFACTURE

A brief outline of a typical manufacturing process will now be given (cf. also Anonymous, 1952; Wolff *et al.*, 1954). Pure cultures of the selected strains of leuconostoc are maintained in the laboratory on sucrose-agar slopes subcultured at 14 day intervals. Yeast extract is also incorporated, as vitamins of the B group are essential for growth. Each culture is incubated at 23°C. for 48 hours and then stored at 4°C. The bacteria are then inoculated on to successively larger slope cultures and finally into 5-litre flasks containing yeast-extract, peptone, various salts and sucrose. These ingredients in varying proportions are the medium used throughout the production of dextran. All media are sterilized at 15 lbs. per sq. inch steam pressure for 30 minutes at exactly neutral pH. These conditions are important because if the sucrose is hydrolyzed no dextran will be produced. When these liquid cultures are growing vigorously several of them are used to inoculate larger seed vessels holding several gallons of medium. These cultures are in turn used for the inoculation of 1000-gallon stainless steel fermenters. After 48 hours the cultures become very viscous from the presence of native dextran. At this stage the dextran may be separated as a gum by precipitation with alcohol. Then sulphuric acid is added either to the whole culture or to a solution of the precipitated gum and the mixture is heated under controlled conditions. The object of this treatment with acid is to hydrolyze the molecules of native dextran which may have a molecular weight of several million, and so to break them down to smaller molecules of about the same size as the plasma

proteins. In this step a wide range of molecular size is produced which must later be narrowed if a satisfactory plasma substitute is to be obtained. The acid is then neutralized, calcium chloride is added and insoluble calcium salts, mainly phosphate and sulphate with adsorbed nitrogenous materials, are separated. The remaining salts are removed by passage through ion exchange resins. Dextran is fractionally precipitated from the clear solution by gradual addition of acetone or alcohol. The larger molecules precipitated first are rejected; after this, further addition of, say, acetone brings down the "middle cut" which is preserved for preparation of plasma substitute while most of the smaller molecules remain behind in solution and do not appear in the product. As in the manufacture of petroleum by distillation, this procedure must be repeated several times to obtain the best product, and a balance of cost against quality partly determines how much fractionation is adopted. After removal of pyrogenic substances by a further adsorption process the dextran is filtered and bottled for use, or spray-dried to a powder for storage or transport.

Several alternatives to various steps in the manufacturing process have been devised. Instead of using large scale growth of leuconostoc the polymerization step may be carried out with the isolated enzyme (Tsuchiya *et al.*, 1953). This enzyme must of course be prepared in a preliminary fermentation on a smaller scale. The depolymerization step may be effected by alkaline or enzymic hydrolysis or by the action of an alternating electrical field (Pautard, 1953), heat, ultrasonic waves or gamma rays (Ricketts & Rowe, 1954). Of these only the thermal (Stacey & Pautard, 1952) and ultrasonic (Stacey, 1951; Lockwood *et al.*, 1951) methods have been used on a production scale. Most dextran is still produced by bacterial fermentation and subsequent acid hydrolysis as described above.

Dextran is available as a 6 per cent dextran solution in 0.9 per cent sodium chloride or as a salt-free solution for specialized applications. Dextran is manufactured and processed as a plasma substitute by various firms. In the remainder of this book, the term "clinical dextran" is used to describe the partially hydrolyzed product fractionated to the degree currently accepted as suitable for use as a plasma substitute, reserving the name "native dextran" for the undegraded bacterial product.

STRUCTURE OF GLUCOSE

Dextran consists of long chains of glucose units. Glucose, $C_6H_{12}O_6$, has the well known formula shown in Figure 1 (*a*) and occurs naturally in the ring form (Figure 1 (*b*)) which is obtained from the former by migration of a hydrogen atom from the OH position at (5) to form an OH group at (1). In Fig. 1 (*c*) the pyranose ring is shown in perspective drawing. Most of the

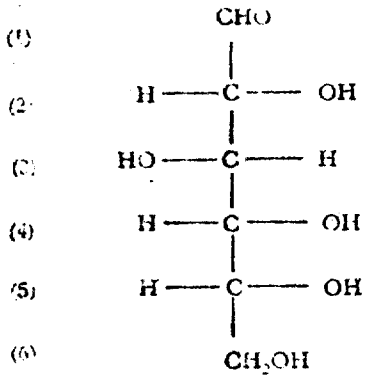


FIG. 1(*a*). D-glucose in its simplest formulation.

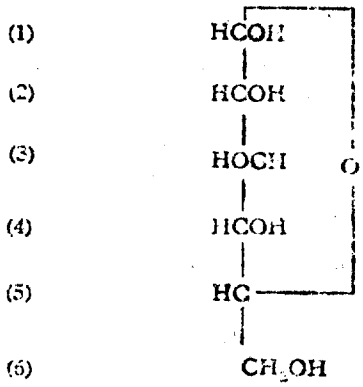


FIG. 1(*b*). Pyranose ring form of D-glucose as derived from 1(*a*). Notice the migration of a hydrogen atom from the OH group of $C_{(5)}$ to the O at $C_{(1)}$.

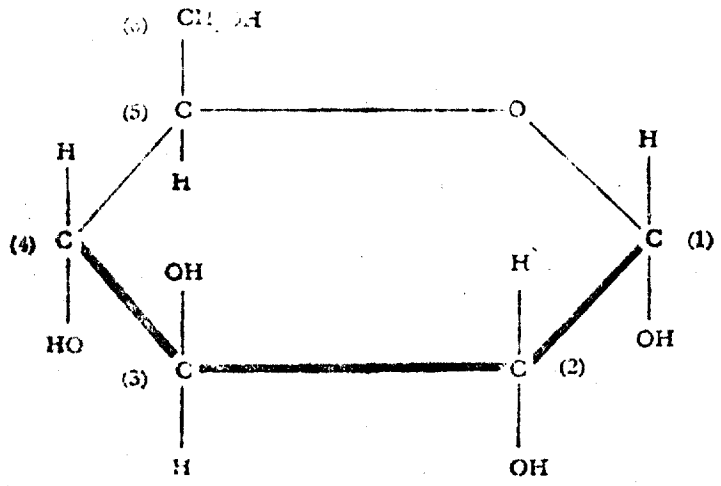


FIG. 1(*c*). Perspective view of pyranose ring form of alpha D-glucose. The [illegible] are shown thickened.

glucose units in dextran are joined with loss of water through positions (1) and (6) by the alpha type of link in which the linking oxygen atom is below the plane of the ring containing the adjacent $C_{(1)}$ as illustrated in Figure 2. The chains of glucose units may also have branch chains joined to them through positions (3) or (4). Polyglucoses other than dextran containing combinations of most of the possible kinds of link are known; for instance in glycogen the glucoses are mostly joined through (1) and (4) but branch chains are joined through (6). It is convenient therefore to define dextran as *a polyglucose in which the majority of bonds linking the glucose units is of the alpha* 1 : 6 *type*, and so having the high degree of dextrorotation already mentioned.

These details of dextran structure were elucidated mainly by the classical method of methylation of the polysaccharide, followed by complete hydrolysis to the constituent methyl glucoses, then separation and identification of these fragments of the original molecule. These methyl glucoses are only partially methylated because the methyl group cannot enter at the positions occupied by main- and branch-chain linkages. This work was reviewed by Stacey & Swift (1948) and establishes the 1 : 6 alpha type of linkage as the principal type in dextran. A 1 : 4 link was at that time suggested as the branch linkage.

Another standard analytical method of carbohydrate chemistry, quantitative oxidation with sodium metaperiodate, leads to an estimate of the ratio of 1 : 6 links to linkages of other types. The periodate ion has the property of oxidizing adjacent CHOH groups to CHO groups, being itself reduced to iodate in the process. This occurs twice in each unbranched 1 : 6 linked glucose unit as shown in Figure 3 with conversion of $C_{(2)}$ to one molecule of formic acid. Where one of the OH groups of a glucose unit (positions (4) or (2)) is the point of attachment of a branch chain, double oxidation does not occur, so that no formic acid is produced and less periodate is consumed. Where the branch chain is attached through the OH group at position (3), no oxidation occurs. Consequently a low yield of formic acid from periodate oxidation is an indication of chain branching in the dextran molecule. These reactions are difficult to carry out quantitatively and their interpretation should be treated with reserve. Branching ratios between three and thirty 1: 6 links per branch link have been reported for dextrans produced by

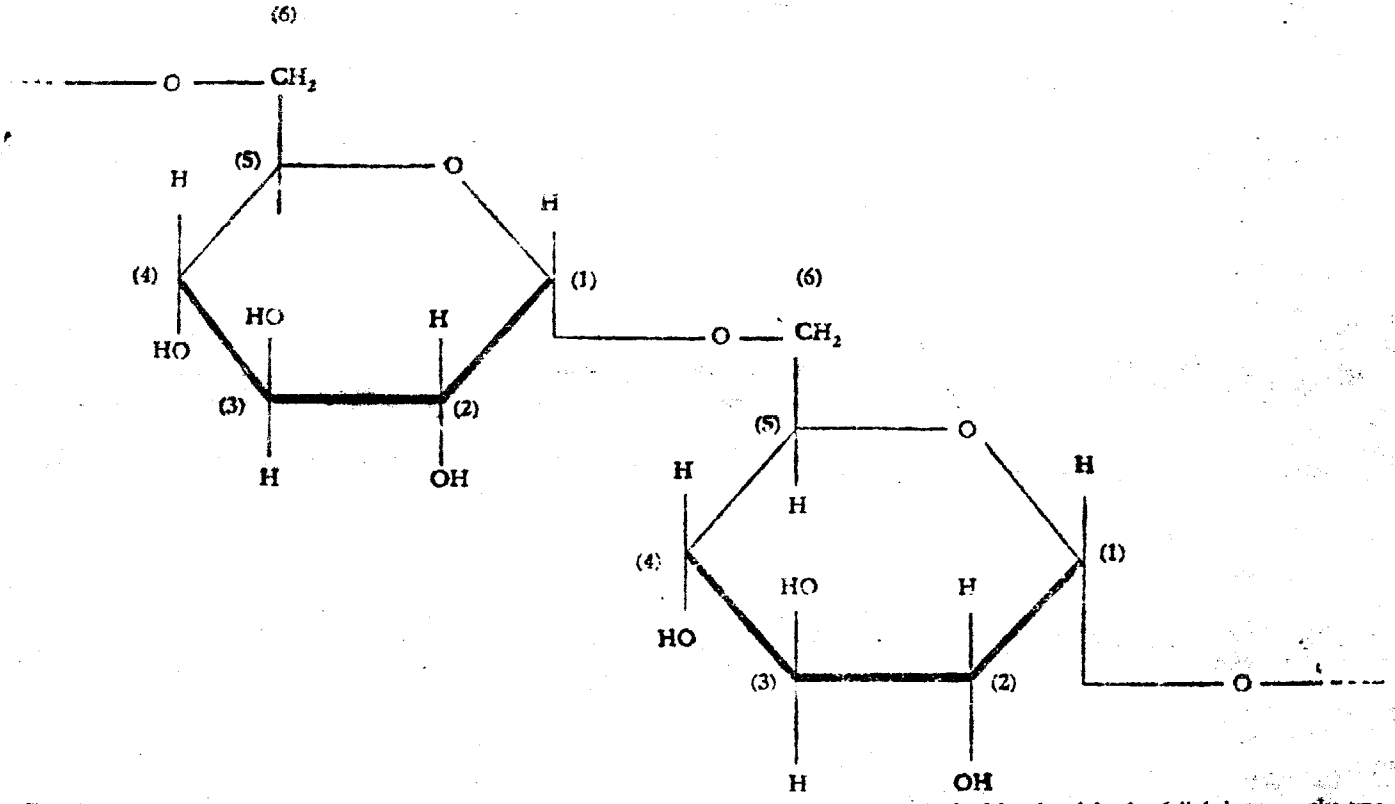


FIG. 2. Two glucose units forming part of the molecular chain in dextran. Dextran is characterized by the alpha 1 : 6 link between the two units. Notice that in the alpha link the oxygen atom lies below the plane of the ring containing $C_{(1)}$. (The C atoms have been designated in this diagram by numerals only for the sake of simplicity.)

various strains of leuconostoc. As mentioned, above where the branch chain is attached through position (3) no oxidation by periodate occurs. Abdel-Akher *et al.* (1952) and, independently,

PERIODATE OXIDATION OF GLUCOSE UNIT IN DEXTRAN

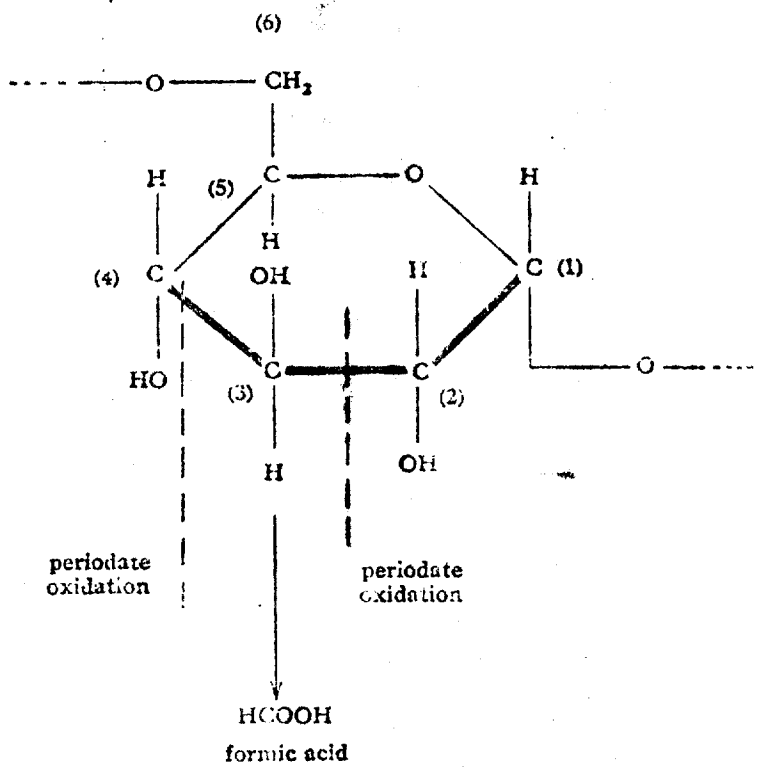


FIG. 3. Oxidation of a glucose unit in dextran by two periodate ions. $C_{(3)}$ is eliminated as formic acid while $C_{(2)}$ and $C_{(4)}$ are oxidized to aldehyde groups.
The colourless form of the dye fuchsin complex with these aldehyde groups to form an insoluble purple complex. This is the basis of the histochemical detection of dextran in tissue sections referred to on page 48.

Lohmar (1952) utilized this fact and obtained positive evidence of the presence of 1 : 3 links in some dextrans. Dextran previously oxidized with periodate was hydrogenated and hydrolyzed. From the resulting mixture of glycols, sorbitol was isolated as its pyridine complex and characterized as the hexa-acetate. This sorbitol could only have come from glucose units in which position (3) was the point of attachment of a branch chain.

Differences in the infra-red spectrum of various dextrans reported by Barker & Melvin (1952) were investigated more thoroughly by Barker *et al.* (1953, 1954*a*) who examined the spectra of a wide range of sugar derivatives and showed that a spectral peak corresponding to the alpha 1 : 3 link occurred in some dextrans.

Barker *et al.* (1952), showed that by methylation, by partial hydrolysis to 3-glucosyl-D-glucose and by periodate oxidation, dextran from a *Leuconostoc sp.* known as *Betacoccus arabinosaceus*, Birmingham strain (Stacey & Swift, 1948) was a highly branched 1 : 6 alpha glucosan in which the branches were almost exclusively of the 1 : 3 type and probably of the alpha form. Full details of this work are given by Barker *et al.* (1954*b*). The partially methylated glucose corresponding to the presence of a 1 : 3 link, 2 : 4 di-O-methyl glucose, was isolated as its crystalline aniline derivative (N-phenyl 2 : 4 di-O-methyl glucosylamine). In view of this and the supporting evidence from chromatography, ionophoresis and infra-red spectra the presence of the 1 : 3 link in this dextran may be regarded as satisfactorily established. The authors suggest that in the five years which elapsed since the work of Stacey & Swift (1948) during which the organism was repeatedly subcultured there may have been a change in strain; alternatively, an impurity in the earlier aniline derivative may have led to a melting point close to that of N-phenyl 2 : 3 di-O-methyl glucosyl amine (which led to the suggestion of the presence of 1 : 4 links).

Thus in any dextran the principal link is the alpha 1 : 6 type and the branch links, if any, may be of the alpha 1 : 3 or the alpha 1 : 4 type. In the present state of our knowledge the possibility of the alpha 1 : 2 or the beta type of link occurring in some dextrans cannot be excluded. Nor is it known whether, for a given branching ratio, the branch chains are relatively long as in Figure 4 (*a*) or relatively short as in Figure 4 (*b*).

The predominant linkage present in clinical dextran depends on the salts present during fermentation, the extent of hydrolysis and above all upon the strain of leuconostoc used. A further complication is that the fractional precipitation with ethanol of some dextrans may result in the separation into fractions free from 1 : 3 linkages and into others with an increased proportion of such linkages (Lohmar, 1952; Jeanes & Wilham, 1952). Prior to 1954, major differences in branching-ratio were present in dextran

of British and U.S. origin. Recently, however, the U.S. strain known as "NRRL–B512" has been mainly used in these and

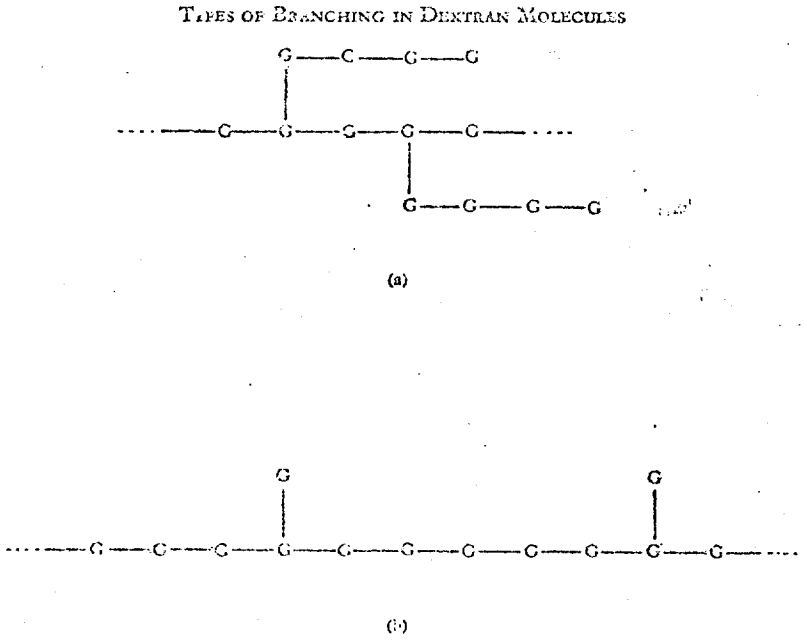


FIG. 4. Schematic structure of two fragments of dextran molecules each having a branching ratio of 5 : 1.("G"=glucose unit).

other countries. The resultant clinical dextran contains comparatively few branches and approximates to a simple unbranched 1 : 6 alpha polyglucose.

POLYMER PROPERTIES

Dextran has a wide dispersion of molecular weight. Such materials can only be described in terms of average molecular weights. Two kinds of average are commonly used, the arithmetic mean or *number average* M_N and *weight average* M_W. The *number average* is the sum of the products of the molecular weight of each species and its corresponding proportion by number in the mixture. For *weight average* the molecular weights are multiplied by the proportions of each by weight. M_W generally has a value between M_N and $3M_N$ and the nearer M_W is to M_N the less the dispersion of molecular weight.

The physical measurements from which these average molecular weights can be calculated are as follows:—

1. *Number average*
   (*a*) End-group assay using radioactive cyanide
   (*b*) Osmotic pressure
2. *Weight average*
   (*a*) Sedimentation in ultracentrifuge; diffusion constant
   (*b*) Light scattering
   (*c*) Intrinsic viscosity

These will each now be briefly discussed.

1 (*a*) *Number average* is most simply obtained by counting the number of molecules in a sample of known weight. Since there is only one reducing end-group per molecule the ratio of the reducing power of glucose to that of dextran is directly proportional to the average number of glucose units in each molecule. The molecular weight is obtained simply by multiplying this ratio by the formula-weight of each unit, 162. The comparison of reducing power may be made by volumetric analysis but to obtain adequate sensitivity a method based on radioactive measurements has been employed. A ^{14}C-labelled cyanide group is combined with each reducing group and the radioactivity due to ^{14}C is measured (Isbell, 1951). The number average obtained by these methods includes all dextran molecules together with any glucose. Traces of glucose are often present since they are difficult to separate from the dextran, so that underestimation of the number average molecular weight of the dextran may occur.

1 (*b*). The osmotic pressure of a solution is proportional to the number of solute molecules under ideal conditions, and is theoretically a good method of measuring number averages. But to obtain ideal conditions with large molecules it is necessary to make measurements at low concentrations, and in aqueous solutions various sources of error, such as surface tension effect, become appreciable with many types of osmometer. Further, with dextran solutions the molecular weight dispersion may lead to the smaller molecules passing through the osmometer membrane and so tend to over-estimation of molecular weight. A new type of osmometer described by Rowe (1953) has been used recently for estimating the molecular weight of dextran.

2 (*a*). *Weight average* molecular weights have been calculated from ultracentrifuge measurements by Ingelman & Halling (1949)

and by Wales *et al.* (1953) for dextran fractions having a much narrower distribution of molecular weight than dextran for clinical use. A very considerable amount of work is involved in such determinations which are therefore unsuitable for quality control work.

2 (*b*). Measurements of the light scattered during its passage through a polymer solution provide data from which weight average molecular weight can be calculated (Brice & Speiser, 1950; Doty & Steiner, 1950). This has been applied to aqueous solutions of dextran in the U.S.A. (Riddick *et al.*, 1954) and in

Relation between Intrinsic Viscosity and Molecular Weight of Dextran (NRRL—B512)

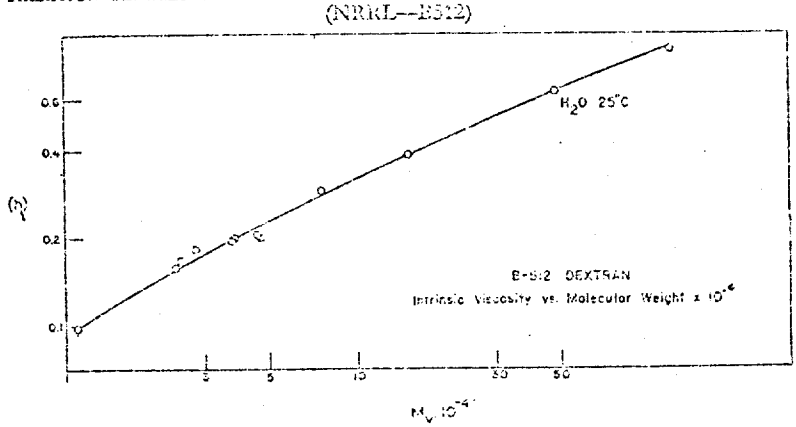


Fig. 5

The intrinsic viscosity $[\eta]$ and molecular weight (viscosity average) M_V, are plotted on logarithmic scales. The resulting relation is approximately linear, corresponding to the empirical relation $[\eta] = 10^{-3}M_V^{\frac{1}{2}}$. The data is reproduced from Wales *et al.* (1953) for partially hydrolysed dextran prepared from *Leuconostoc* strain NRRL-B512.

Britain. Dust must be carefully excluded because particulate matter contributes to the molecular weight. It is believed that light scattering measurements would be valuable for quality control of dextran.

2 (*c*). The intrinsic viscosity of dextran is the simplest property related to molecular weight and can be measured in any laboratory (see Appendix I). For this reason it has been universally used in factories and the early stages of clinical research. Intrinsic viscosity $[\eta]$ is related to molecular weight M by the equation

$$[\eta] = KM^n,$$

where K and n are parameters having different values for dextrans

differing in branching ratio. For molecular weights in the range 40,000 to 300,000, Ingelman & Halling (1949) quote

$$[\eta] = 8.2 \times 10^{-7} M + 0.18$$

while for a different dextran Wales *et al.* (1953) quote for the range 20,000 to 250,000

$$[\eta] = 10^{-3} M^{\frac{1}{2}}$$

The actual values found are plotted in Fig. 5.

TABLE I

Molecular Weights of Dextran Fractions
(dextran from *L. mesenteroides* NRRL—B512)
Data from Wales *et al.* (1953)

Intrinsic Viscosity dl/g. in water at 25°C.	M_N	M_W	M_V
0.097	—	11,000	11,000
0.158	—	24,000	24,000
0.166	23,100	25,000	25,000
0.183	28,500	28,000	28,000
0.196	—	37,500	37,500
0.197	—	41,500	38,300
0.207	41,000	47,000	45,600
0.202	38,000	50,000	46,300
0.290	64,000	76,000	76,000
0.384	95,000	170,000	153,000
0.633	240,000	540,000	480,000
0.886	—	1,700,000	1,200,000

M_N and M_W are explained on p. 16. M_V, the 'viscosity average', is a further kind of average related to intrinsic viscosity by the equation $[\eta] = 10^{-3} M_V^{\frac{1}{2}}$. A plot of $[\eta]$ against M_V on logarithmic scales is given in Fig. 5 and this provides the simplest indication of molecular weight for experimental purposes. This formula applies to dextran produced by the NRRL—B512 strain of *Leuconostoc* and may need modification for other types of dextran. For fractions the viscosity average approximates to the weight average below about 100,000. At higher molecular weights the deviation of viscosity average from weight average may be ascribed to the known heterogeneity of the "fractions" as well as to the failure of the linear relation between log $[\eta]$ and log M beyond 250,000.

Wallenius (1954) in his paper on the renal excretion of dextran quotes a similar relation between intrinsic viscosity and molecular weight independently determined by K. Granath. Typical average molecular weights and intrinsic viscosities of dextran fractions are quoted in Table I. Table II shows some properties of clinical dextrans currently in use.

TABLE II

Properties of Clinical Dextrans

Type and year produced	*Optical Rotation*	*Branching Ratio*	*Intrinsic Viscosity*	M_N	M_W	*Relative Viscosity 6% soln.*	*Proportion excreted in urine (rabbit, 24 hours)*
British (1953)	202° to 205°	1:5 to 1:9	0.32 to 0.37	90,000	—	5	15%—25%
U.S. (1952)	+198° to 200°	1:20	0.18 to 0.28	—	75,000 ±25,000	3	30%—50%
British (1954)	+198° to 202°	1:10 to 1:20	0.32 to 0.37	80,000 to 90,000	170,000 to 250,000	5	10%—20%
U.S. (1954)	+195° to 198°	1:20 or more	0.18 to 0.28	50,000 to 60,000	70,000 to 80,000	3	35%—45%

In fact there has been appreciable variation from batch to batch and from manufacturer to manufacturer, but an attempt has been made to include representative figures. Currently produced British dextran exhibits branching ratios more similar to those of U.S. dextran since similar strains of organism (NRRL—B512) are now being used. Dextran is prepared in several other countries but it has not been possible to compile such detailed information about these dextrans.

The shape of a high polymer molecule in solution cannot be determined simply. On the basis of their ultracentrifuge data Ingelman & Halling (1949) favoured an elongated form, assuming that the molecules were not hydrated. As they pointed out this is almost certainly not the case in solution. Ogston & Woods (1954) re-examined this problem and concluded that in solution dextran molecules are highly hydrated and nearly spherical.

DETERMINATION OF DEXTRAN

Considering firstly the estimation of pure dextran in water, the solution can be freeze-dried to obtain a porous solid which can readily be freed from moisture at 100°C. under 0.1 mm. Hg

pressure (see Appendix I). The physical form of the sample is extremely important in drying to constant weight. Riddick *et al.* (1954) report difficulty in removing the last 0.3 per cent of moisture (determined by Karl Fischer reagent) from alcohol-precipitated dextran powder by drying in an oven at 110°C. Dry dextran can be weighed and used to prepare a solution of known concentration to serve as a standard for other determinations. The optical rotation of this solution may be measured and the specific rotation, $[\alpha]_D^{20}$, of that particular dextran calculated. Subsequently, determinations can be made polarimetrically on solutions of the same dextran in water, physiological saline or aqueous solutions containing less than 25 per cent v/v of alcohol or acetone which may be encountered in fractionation procedures. Alternatively measurements of refractive index may be used but are less sensitive and affected greatly by acetone and alcohol.

Dextran may be hydrolyzed to glucose and the glucose determined by any standard procedure. Acid hydrolysis followed by cuprimetric determination of reducing power yields 95 to 105 per cent of the expected amount of glucose depending on the concentration of dextran in the sample. Some decomposition of glucose to other reducing substances is inevitable. Hydrolysis of a 10 ml. sample containing about 0.5 per cent dextran in *N* sulphuric acid in a test tube under reflux on a boiling water bath for four hours followed by neutralization and determination of reducing power by the method of Schaffer & Hartmann (1921) gives apparently 100 per cent of the theoretical yield of glucose.

Alternatively a colorimetric method may be used and it is convenient to rely on the heat of mixing an aqueous sample with concentrated sulphuric acid to hydrolyze the dextran and convert the resulting glucose to its coloured complex with "anthrone" or similar reagent (Roe, 1954). A convenient reagent, resorcinol 4 : 6 disulphonic acid, which is stable in concentrated sulphuric acid solution, has been described by Lunt & Sutcliffe (1953). Calibration with pure dry dextran avoids the uncertainty about glucose decomposition products which confounds methods depending on reducing power.

A novel method for estimating dextran in plasma was devised by Hint & Thorsen (1947) who precipitated a copper complex of dextran from aqueous solution and determined the copper remaining in the supernatant. This is the only method which provides

more or less specific separation of dextran from other sugars and polysaccharides. It could well be followed by colorimetric determination of dextran in the precipitate which would probably be the best method for very small amounts of dextran in biological fluids. Bloom & Wilcox (1951) have devised an alternative method for dextran in plasma in which strong alkali is used to retain proteins in solution while dextran is precipitated with ethanol and subsequently estimated with anthrone.

Dextran in human urine may be estimated by any of the foregoing methods. Polarimetry is the quickest method, but cannot be used if the diluted sample is too highly coloured; otherwise hydrolysis is recommended since corrections for sugars but not for mucopolysaccharides are easily applied. Colorimetric analysis, which would include sugars and mucopolysaccharides, may often be adequate in clinical laboratories. Jacobsson & Hansen (1952) even describe a simple turbidimetric method for the determination of dextran in blood and urine.

Some notes on useful laboratory methods are given in Appendix I.

Chapter III

Behaviour of Dextran in the Body and Effect on Body Constituents

Effects on Circulation, Excretion, Storage and Metabolism

THE aim of therapy with dextran or other plasma substitute is to sustain the circulatory volume by giving a colloid which will exert an osmotic pressure effect similar to that of plasma protein and remain in the circulation until replaced by natural colloids. In practice, plasma substitutes suffer varying amounts of loss by excretion, by metabolism, by passing into the extravascular fluid and by storage. In the case of dextran, the rate of metabolic breakdown is not rapid, so that the main losses during the crucial first 24—48 hours of shock treatment are those resulting from diffusion into the tissues and excretion via the kidney. Both these losses mainly concern the smaller molecules.

In their pioneer studies Grönwall & Ingelman (1945) demonstrated the main physiological effects of dextran. They showed that intravenous injection into previously bled animals restored the circulatory volume and blood pressure and that the dextran concentration in the blood declined gradually over a period of days, some of the material being found in the urine. These results have been confirmed and amplified by other workers. Corresponding findings in experiments on human volunteers given dextran after experimental haemorrhage are summarized in Fig. 6 (Hanmarsten *et al.*, 1953).

The detailed effects on circulatory dynamics of infusion of dextran have been demonstrated by cardiac catheterization of patients undergoing surgical operation. The right auricular pressure increases as dextran is infused and the cardiac output and blood pressure rise correspondingly (Bull *et al.*, 1951). Similar observations made in America on normal volunteers confirmed these findings. Blood volume was found to increase by an amount similar to the quantity transfused and the cardiac output rose by about a third after the infusion of one bottle of dextran (Witham *et al.*, 1951). Other studies explored the effects of fractions of different molecular sizes showing, as would be expected, that the large molecules are less rapidly lost and sustain

the circulatory volume longer than the small molecules (e.g., Wasseman & Mayerson, 1954).

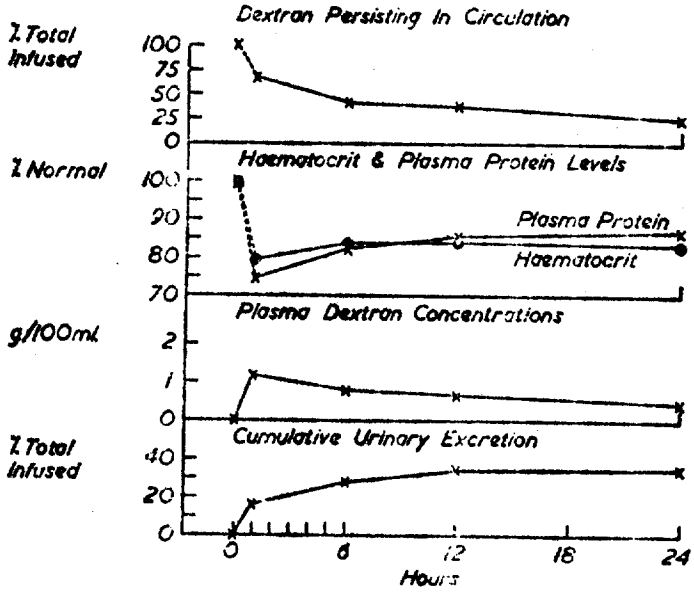


FIG. 6. Effect of administration of 1 litre of 6 per cent U.S. dextran to volunteers from whom 1 litre of blood had been previously withdrawn (mean of results on 8 volunteers). Redrawn from data of Hammarsten *et al.*, 1953.

Detailed studies of the excretion of molecules of dextran of different sizes have been made by Ricketts *et al.*, (1950) and clearance measurements by Brewer (1951). From renal clearances obtained in rabbits, Brewer concluded that dextran is mainly excreted by glomerular filtration, any tubular excretion or reabsorption being relatively small in amount. His results suggested a range of glomerular permeability rather than a sharp level of filtration or retention, and he was able to express the excretion of the different sizes in terms of their probable "half-life" in the body; for instance, dextran fractions of about 25,000 molecular weight were calculated as having a "half-life" of 1.2 hours, while a fraction of 38,000 molecular weight was calculated to have a "half-life" of 4.8 hours. Wallenius (1954) found similar values in dogs and showed that the renal clearance of molecules less than 10,000 molecular weight was almost 100 per cent of the glomerular filtration rate. Larger molecules had progressively lower

clearance values until at about 50,000 molecular weight and above, no appreciable excretion was found. The largest molecules of dextran found in the urine after infusion of normal adults was about 46,000, but three patients with burns excreted material up to about 56,000 molecular weight. In other states of increased glomerular permeability such as glomerular nephritis, permeability to dextran may be further increased, and molecules as large as 104,500 molecular weight may appear in the urine in these conditions. A useful table, summarizing the different rates of excretion of the different sizes of molecules of plasma substitutes including dextran, is given by Gropper *et al.* (1952).

As most of the smaller material is lost rapidly, high dextran concentrations may be found in the urine when large transfusions are given. Such urine is noticeably viscous, and may contain 10 g. or more of dextran per 100 ml. It has not been shown that this causes harm, though by analogy with other carbohydrate substances all possibility of damage cannot easily be excluded and transient histological changes similar to those following sucrose infusion have been reported in animals. After the first few hours during which most of the smaller molecules are lost, the rate of excretion falls rapidly, so that half of the total excreted appears within 8 hours or so, and little further can be found in the urine after 48 hours.

Satisfactory clinical material is excreted to a total of up to about 25 per cent (see p. 57), but the levels obtained in the blood do not account for all the remaining material. This is to be expected if there is loss into the extravascular fluid, and early disappearance from the circulation is particularly marked when small molecular material is infused (Semple, 1954). In their study on dogs, Wasserman & Mayerson (1952) showed that the ratio of the levels of dextran in plasma and lymph remained steady after initial equilibration, and that for clinical dextran this ratio was higher than that for either albumin or globulin. Smaller molecular material (molecular weight 19,000), by contrast leaked out from the capillaries so readily that after a short time levels in the lymph were higher than those in the plasma due to the gradient established by the high renal clearance of such small molecules from the plasma. In clinical use, plasma levels may be further affected by losses of material into the lesion. When dextran is used in the treatment of burn shock, volumes of 6 per

cent dextran equal to about the plasma volume of the patient commonly yield levels of about 2 g./100 ml. at the end of a transfusion period lasting perhaps eight hours. The levels so achieved slowly fall and, with British clinical material, the rate of fall is about one-third decrement per 24 hours. This implies that in a patient with a post-transfusion level of 2 g./100 ml. there will be a persistent dextran level of rather less than 1 g./100 ml. 48 hours later. During this time the plasma colloid level of protein plus dextran tends to remain constant, the dextran substituting for protein in maintaining a colloid osmotic pressure and circulatory volume. Experiments have shown that the primary effect of dextran is a dilution of the total circulating protein which remains unaltered in amount. This may not be true if large amounts of dextran are given when the blood volume is already normal. In this case plasma containing both protein and dextran is probably lost from the bloodstream until the circulatory volume is corrected (Wasserman & Mayerson, 1954).

In common with many other macromolecular substances, dextran is found to be stored temporarily in various organs. In the rabbit dextran can be detected and estimated chemically in the liver, kidney, spleen and lymph glands up to two or three weeks after an injection (Bull *et al.*, 1949). In a subsequent study, using a serological method, Maycock was still able to find small quantities in certain tissues up to 12 months after injection (see p. 46). Friberg *et al.* (1951) examined the storage following infusion of fractions of different molecular size and found that the amount in the kidney was greatest with the smaller molecular fractions, but these workers were unable to decide whether the presence of dextran in the tubular cells of the kidney represented reabsorption, excretion or storage. After the severe procedure of repeated daily injection for 100 days into rabbits, only moderate storage in liver, spleen and reticulo-endothelial system was found using chemical methods of detection (Friberg *et al.*, 1953). Studies of renal function after administration of dextran to human volunteers revealed no marked effects upon glomerular filtration rate or tubular reabsorption (Fleming *et al.*, 1951). Similarly hepatic function as measured by hippuric acid and phosphatase tests and bilirubin and urobilinogen determinations has been found to be unaltered (Bohmansson *et al.*, 1946). It thus appears that a small proportion of infused clinical dextran remains for

time in certain organs, particularly those concerned with the reticulo-endothelial system, but the material so retained gradually disappears, apparently without having caused harm.

In short-term experiments almost all of the dextran transfused can be accounted for either in the circulation, in the urine or in the tissues, but long-term studies show that the material not excreted, which presumably contains large molecules, slowly disappears and traces of it can be detected serologically in the urine over several months (Lorenz & Maycock, unpublished observations). The presumption that some metabolic breakdown of dextran occurred was supported by Ingelman (1947) on the grounds that the rate of disappearance was increased by administration of thyroid to experimental animals. Nevertheless, incubation of dextran with blood or with organ extracts has failed to demonstrate any breakdown (Swanson & Cori, 1948; Bull *et al.*, 1949; Engstrand & Åberg, 1950). The possibility that metabolism may occur within the gut has been considered by many workers. The ability of anaerobic organisms from the human intestine to hydrolyze dextran (Lehre & Sery, 1952) explains the observation of Åberg (1953) that human faeces will degrade dextran. Various authors have shown that dextran can be detected in the gastric juice after an intravenous injection of dextran both in animals and man (Engstrand & Åberg, 1950; Gray *et al.*, 1951; Burson & Bloom, 1951; Troell & Åberg, 1952) but the significance of this route of excretion of dextran has not yet been established. Apart from the observations of Engstrand & Åberg (1950), the amounts of dextran recovered from the gut have formed only a small part of the dose of dextran given intravenously. Bloom & Wilhelmi (1952) have reported that the duodenal mucosa of rats may contain an enzyme capable of hydrolyzing dextran; if such an enzyme is present in the mucosa of other species, it is possible that any dextran excreted into the gut is rapidly hydrolyzed, so that a high concentration would not then be found.

Recent work with radioactive dextran (Scully *et al.*, 1952) has confirmed that some metabolic breakdown occurs since radioactivity can be detected in respired carbon dioxide and urinary carbonate. Cargill & Brunner (1951) first described this finding in mice and similar results have been obtained in dogs, about

25 per cent of the infused dextran being recovered as carbon dioxide and almost all being accounted for either by excretion or metabolism (Terry *et al.*, 1953). Gray (1953) using radioactive dextran in mice, showed that the half-life of the material remaining after the initial period of rapid renal excretion was about six days and that the carbon, after degradation of dextran, was incorporated into various body tissues. These experiments need further confirmation, particularly with larger molecular sizes of dextran, but it appears probable that similar breakdown will be found to account for the final disappearance of clinical dextran administered to man (Hellman, 1951). If so, it would seem appropriate for its use as a plasma substitute that dextran should be retained in the body during the acute phase of circulatory volume deficit but should undergo slow metabolism and so presumably give rise to no permanent storage phenomena.

EFFECT ON THE FORMED ELEMENTS OF THE BLOOD

The recipients of the early infusions of dextran were naturally carefully scrutinized to see whether untoward effects were produced (Grönwall & Ingelman, 1945). No systematic alteration in the numbers or properties of white blood corpuscles or platelets was found, but one definite effect on erythrocytes was noted. Blood drawn at any time up to about 1—3 days following a large infusion of clinical dextran showed an increase in the erythrocyte sedimentation rate above normal. The effect has subsequently been fully investigated; it is greatest at the time when dextran concentration is highest (i.e. usually at the end of infusion) and gradually becomes less. The rise in E.S.R. is accompanied by excessive rouleaux formation (Grönwall & Ingelman, 1945; Bull *et al.*, 1949), which is in fact the basis of a raised erythrocyte sedimentation rate in all circumstances other than anaemia. The effect is readily reproduced by adding dextran to blood *in vitro* (Ingelman & Halling, 1949; Thorsen & Hint, 1950; Hardwicke *et al.*, 1950; Hardwicke, 1951) and by such tests it can be shown to depend on two main factors—the concentration of dextran present, and its molecular size.

If a standard concentration of dextran is used (e.g. 1 g./100 ml.) increasing molecular sizes of dextran produce greater and greater rises in the E.S.R., until molecular weights of the order of 1,000,000 are attained. The E.S.R. is then less affected, but microscopic

examination, shows that marked rouleaux formation is still present; for, solutions of dextran predominantly of these very high molecular weights are so viscous that the fall of even large rouleaux through the solution is slowed to approach the rate of fall of smaller rouleaux through less viscous solutions. For this reason, Hardwicke & Squire (1952) as part of a comprehensive study of the factors concerned in the erythrocyte sedimentation rate used corrections allowing for the viscosity of the suspending fluid. They also chose (like various other authors) to use the *maximum rate of fall* of the erythrocyte as the best index of rouleaux formation, ignoring both the early part of the sedimentation test (when rouleaux have not fully formed) and the later part (when erythrocytes may become "packed" in the sedimentation tube). In this way, a regular empirical relation was found with different dextran fractions, as well as with other plasma substitute solutions, between erythrocyte sedimentation and concentration of colloid, the effect increasing approximately as the square of the concentration. Little or no effect of this kind can be detected with dextrans of very low molecular weights (less than 30,000), but the effect is appreciable with dextrans of the range of molecular weights (greater than 100,000) usually present in clinical preparations. One issue remains uncertain. It has been claimed (Thorsen & Hint, 1950) that in the presence of small molecules of dextran, the larger molecules produce less rouleaux formation and a smaller effect on the erythrocyte sedimentation rate. Alternative explanations for this finding can be put forward, and it seems doubtful if the presence of small dextran molecules has any effect on rouleaux formation. Thorsen has made a valuable contribution to these studies by demonstrating clearly in cinematograph records that rouleaux formation occurs within the blood stream of experimental animals given dextran.

The significance of the increased tendency to form rouleaux in the blood of the recipient of an infusion of dextran is not easily evaluated. In practice, no harm has been shown to result from this effect (see Chapter V). Certainly, equally large rouleaux-forming tendencies occur in the course of many infections and other diseases as the result of spontaneous increases in plasma fibrinogen and other large endogenous molecules. A theory of the deleterious effects of "blood sludging" has been put forward

(Knisely *et al.*, 1947) and marked separation of erythrocytes from plasma noted *in vivo* in overwhelming infection (e.g. fatal malaria), but the causal relationship (as opposed to the association) between marked rouleaux formation and serious complications or fatal outcome of such disease-states one may consider to be undemonstrated. Increased intravascular adhesiveness and aggregation of erythrocytes and sometimes clumping of the platelets, accompanied by slowing of the circulation has been recorded in the hamster (Cullen & Swank, 1954) following meals high in fat content and after injection of large molecules such as dextran. Dextran (molecular weight 210,000) was used, though unfortunately the dosage is not reported. Studies on the brains of these animals after trypan blue administration showed a diffuse staining in some instances after dextran administration. At present, therefore, the rouleaux-promoting effect of dextran and other plasma substitutes may be regarded as an undesirable property, associated with no definite untoward results, but one to be kept in mind. This cautious attitude is even more desirable when treating conditions associated with a raised erythrocyte sedimentation rate (e.g. in the nephrotic syndrome with increased fibrinogen and α_2-globulin levels); the dosage of plasma substitutes may then with advantage be more strictly limited. Proposals for including higher molecular weight ranges than those already present in transfusion dextran should also be very carefully scrutinized (see Chapter IV). It has indeed been claimed that a plasma substitute free from erythrocyte sedimentation rate raising effects could be made from dextran, since molecular weights just above the renal threshold do not show any marked effect on erythrocytes. At present, however, this assertion is somewhat academic, since economic considerations, as already noted (p. 10), impose limitations on the fineness of selection of molecular weight ranges in mass-produced material.

BLOOD GROUPING AND COMPATIBILITY TESTS AFTER ADMINISTRATION OF DEXTRAN

The rouleaux forming effect of dextran has an important practical consequence. If serum from the recipient of an infusion of clinical dextran solution has to be used in a compatibility test, the contained dextran may cause the donor's red cells to form rouleaux, thus rendering the detection of agglutinates of cells

difficult or impossible. The formation of rouleaux depends mainly upon the concentration of dextran in the serum and the molecular weight of the dextran, as already noted.

Using American dextran solution, which has a lower mean molecular weight than British dextran, Roche *et al.* (1952) found that satisfactory compatibility tests could be performed after the administration of as much as 3,000 ml. of 6 per cent dextran solution to convalescent patients, and in the presence of a dextran concentration as high as 3.3 g./100 ml. in the recipient's serum. Similarly, these authors found no interference with compatibility tests performed with the serum of patients in "shock" immediately after the infusion of as much as 1,500 ml. of 6 per cent dextran solution. Roche *et al.* (1952) also state that the blood grouping (ABO and Rh) of the red cells of recipients of dextran solution was not affected by infusions or dextran plasma levels of the above magnitude. It is presumed that these authors did not wash the red cells before grouping.

Dextran prepared to the British specification has a higher mean molecular weight, and if present in the serum in concentrations greater than about 1.0 g./100 ml. may cause rouleaux formation and so prevent satisfactory compatibility tests from being performed. Dilution of the recipient's serum with two or three volumes of saline will prevent the formation of rouleaux, but may cause very weak, but true, agglutination to be missed, or the presence of an incomplete antibody to be overlooked. However, use of the indirect Coombs technique will reveal the presence of an incompatible incomplete antibody (Bull *et al.*, 1949).

Marston (1954), reporting 14 cases, suggests that cross-matching of blood for patients who have received dextran should be carried out in the usual way, since in her experience difficulty is by no means always caused by the presence of dextran in the recipient's serum; she recommends the use of an albumin technique described by Dodge (1952) for those samples that cause rouleaux formation.

Nevertheless, whenever a patient is to be given an infusion of dextran of high average molecular weight, it would still seem wise to withdraw a sample of blood before the dextran is given, so that a dextran-free specimen of serum will be available if a compatibility test has to be done. This simple precaution can be taken with little trouble, and ensures that the specimen will

not contain a substance which may complicate the performance of the compatibility test.

Glycine and sodium salicylate have been used for the suppression of rouleaux formation caused by other plasma substitutes (Koop & Bullitt, 1945; Inokuchi, 1950). However, Crawford & Mollison (1953) using suspensions of donor red cells in glycine solutions (6 g. to 24 g./100 ml.) found that the rouleaux-forming properties of serum containing high concentrations (3 g./100 ml.) of British dextran were weakened but not abolished. Likewise, the use of sodium salicylate (1.5 to 6.0 g./100 ml.), although having a more pronounced inhibitory effect than glycine upon rouleaux formation, was found to interfere with specific agglutination so that the presence of weak incompatible antibodies may be missed. They concluded that neither glycine nor sodium salicylate can be considered suitable for routine use to prevent rouleaux formation in compatibility tests on serum containing dextran of high average molecular weight in concentrations greater than about 1.0 g./100 ml.

The presence of dextran in a patient's serum need never interfere with the blood grouping of his cells, since these can be readily freed from dextran by washing three times in normal saline.

INTERACTION BETWEEN DEXTRAN AND PLASMA CONSTITUENTS

There is no knowledge of any interaction between dextran and plasma constituents within the body. The basic approach to this question would seem to be by admixture of dextran and plasma (or individual constituents of plasma) *in vitro*, noting the nature of any interaction found to occur, and then by searching for the consequences of such effects after infusions. At present, a start has been made in this direction by the discovery that precipitates form when dextran is mixed with fibrinogen (Ricketts, 1952). This can occur with dextran molecules of all molecular sizes likely to be useful as plasma substitutes; confirmatory work (Fletcher *et al.*, 1952) has shown that these precipitates with fibrinogen form not only with dextran from various strains of organism, but also with other colloid solutions which might be used as plasma substitutes. Under certain circumstances, fine precipitates can also be found in mixtures of dextran with some specimens of serum, showing that at any rate some serum proteins can act in the same way as fibrinogen.

The fibrinogen-dextran precipitation has not been considered likely to have a serious effect within the body, since fairly high concentrations of dextran are needed (at least 1.5 g./100 ml. final concentration) to precipitate physiological concentrations of fibrinogen, while considerably higher concentrations are needed if the precipitate is to occur at body temperature, and not only after cooling the mixture to 4°C. Even under conditions favouring precipitate formation, relatively small proportions of both dextran and fibrinogen are lost from the solution (e.g. 10—15 per cent). On redissolving the precipitate with saline, the fibrinogen appears to be substantially unaltered, since it still readily forms a clot with thrombin.

It is true that interaction between dextran and plasma constituents could conceivably occur without visible precipitate formation. Major effects should be detectable by osmometry—for example, when iso-osmotic solutions of dextran and plasma are mixed together, the mixture might show a lower osmotic pressure. If this indeed occurs, the magnitude of the effect is quite small, since osmotic pressures of such mixtures are within 10 per cent of that of the separate components (Rowe, 1954). Similarly, no obvious interaction is suggested by viscosity measurements of mixtures, at least in the standard type of Ostwald viscometer. Interaction has been claimed by Russian research-workers (Rozenfeld & Plyshevskaya, 1954) to be demonstrable in strong solutions of dextran and fibrinogen (or other plasma proteins) by measurement of ultra-violet absorption spectra. Further work on these lines is indicated, and light scattering techniques might be expected to be particularly sensitive for this purpose, since these measurements are heavily influenced by the presence of large aggregates.

In vivo, serious interaction might be expected to be manifest following dextran infusions in one of two ways, namely by an excessive fall in concentration of certain plasma constituents, or by the accumulation of deposited material in the form of emboli. By the former kind of test, no excessive fall of concentration has been noted. All plasma constituents are diluted in concert with the fall in haematocrit during the phase of plasma volume expansion, the concentrations returning gradually to pre-infusion levels as the dextran is removed from the circulation: isolated observations on patients suggest an even more rapid rate of

fibrinogen level. Adant (1954) has examined the effects of dextran injections on the blood fibrinogen level of dogs. In normal animals a slight fall in fibrinogen level was usually observed for three to five hours after the injection. In hepatectomized animals a much greater fall occurred, e.g. a reduction of 50—80 per cent. Such falls were not observed in hepatectomized animals injected with saline. This suggested that dextran does in fact increase the consumption of fibrinogen *in vivo*, but that the effect is minimized by activity of the liver in intact animals. Emboli have not been reported. It is possible that some of the uptake of dextran by the reticulo-endothelial system is from removal of finely particulate dextran-fibrinogen complexes, especially after administration of large amounts of dextran. This might explain some loss of dextran from the circulation not accounted for by urinary excretion (see p. 27). But so far no harmful effects attributable to the formation of dextran-fibrinogen complexes have been observed.

MEASUREMENTS OF PHYSICAL PROPERTIES OF SERUM-DEXTRAN MIXTURES

As mentioned on p. 3, the physical properties of serum-dextran mixtures (e.g. in 50 : 50 amounts) are not in every case simply the mean of the value for each separately. For example, the colloid osmotic pressure of 6 per cent dextran may be 75 cm. water, of serum containing 6 g./100 ml. protein 35 cm. water, and the colloid osmotic pressure of an approximately 50 : 50 mixture is only 48 cm. (not 55 cm.). The reason for this discrepancy appears mainly to lie in the marked curvature of the line relating colloid osmotic pressure to concentration both for serum and more especially for dextran (Fig. 7*a*). This departure from the physical "ideal solution" is also marked for the viscosity-concentration relationship with dextran (Fig. 7*b*). The degree of curvature varies somewhat for different dextran solutions, and in general appears to be greater for dextrans of higher molecular weights. In consequence, useful osmotic effects are obtainable even from dextrans of molecular weights appreciably greater than those of the main serum protein fractions, though of course such solutions may be undesirable on other grounds. The anomalous osmotic pressures of albumin-dextran mixtures (Wales *et al.*, 1954) are similar in kind to those found with serum-dextran mixtures.

The main interest of the properties of serum-dextran mixtures lies in the prospect of being able to make useful predictions of the effect within the body of any given dextran concentration, and so, of deciding on rational grounds, the correct dosage required in any clinical situation. As a first approximation, again referring to Fig. 7*a*, it can be seen that iso-osmotic solutions of dextran and serum show no change of colloid osmotic pressure on mixing. For example, serum containing 7 g./100 ml. serum protein had a colloid osmotic pressure of about 43 cm. water, as had the dextran specimen at 4.2 g./100 ml. concentration. A 50 : 50 mixture of these two specimens, having a total colloid concentration of about 5.6 g./100 ml. also had a colloid osmotic pressure of about 43 cm. water, i.e. the same as that of each constituent. . This finding might suggest that a subject losing one-half the plasma volume into a burned area (say 1.5 litres of plasma) would require 1.5 litres of 4.2 g./100 ml. dextran solution, or 1.05 litres of 6 per cent dextran (and perhaps the appropriate amount of extra fluid). This view accords with the known fact that 6 per cent dextran is hyper-oncotic (i.e. has a higher colloid osmotic pressure than normal serum). Fig. 7*b* also demonstrates that the relative viscosity of the kind of mixture circulating after such large infusions is likely to be about 2.9, as compared with 1.6 for normal serum (see Fig. 7*b*) and 5.2—the viscosity of the 6 per cent dextran solution.

Such arguments, though interesting theoretically, are unfortunately not yet applicable to precise dosage calculations in practice, because insufficient is known about the behaviour of dextran in the body and about physiological responses under various circumstances to large colloid infusions. A fair proportion of the dextran administered is lost rapidly into the urine (see p. 25) and as this is of smaller average molecular weight than the material infused, some departure from the curves shown in Fig. 7*a* is likely to be found in the intravascular mixture of dextran and serum protein in the period after infusion. It is also not fully established to what extent the body reacts to colloid infusions "iso-osmotically" or "iso-volumetrically" (Chinard *et al.*, 1954). In general, a constant colloid osmotic pressure is probably maintained when infusions are given early to patients with plasma volumes diminished by plasma or blood loss, but the effects may differ considerably if enough time has elapsed for haemodilution

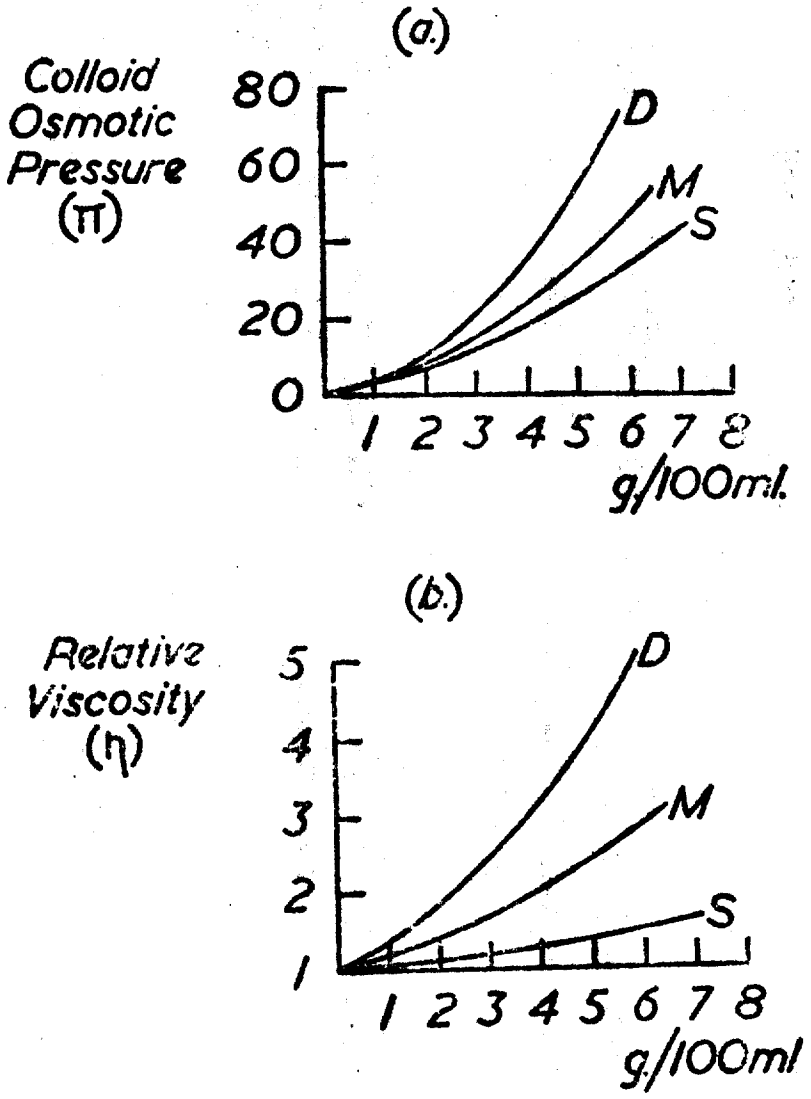


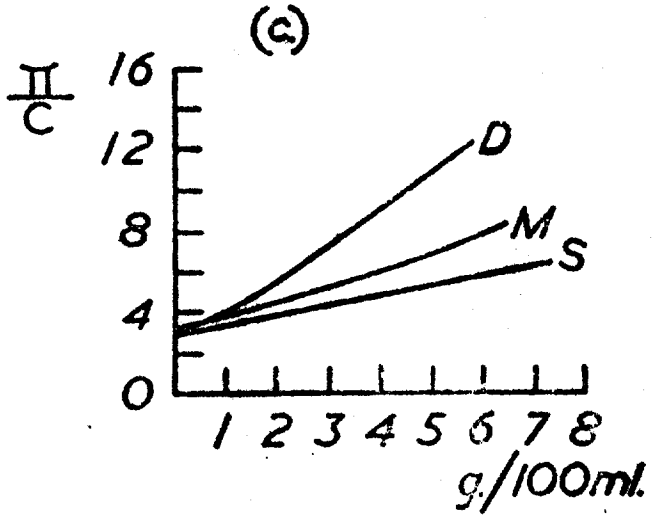
FIG. 7

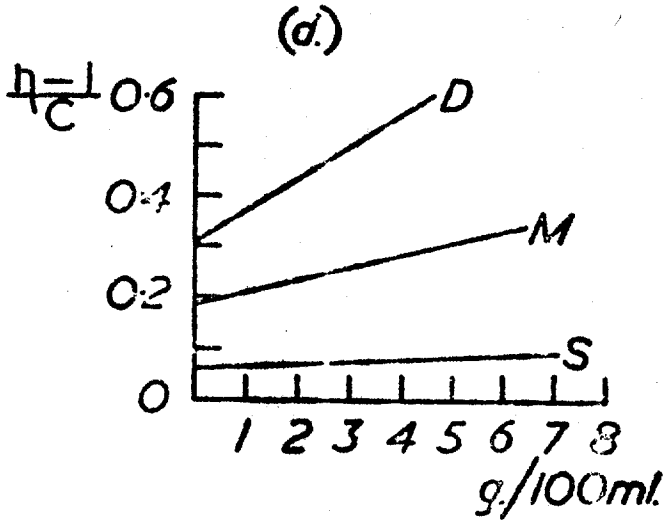
PHYSICAL PROPERTIES OF DEXTRAN-SERUM MIXTURES

D = dextran (British transfusion type)
S = serum
M = mixture consisting of approximately equal parts of dextran and of serum proteins by weight.

(*a*) Colloid osmotic pressure (π) in cm. water, and
(*b*) Relative viscocity (η), for various concentrations of colloid.

As these relations are curved, care must be used in calculating the properties of a mixture from those of its components (see text). (*c*) and (*d*) are derived from





(*a*) and (*b*) respectively and illustrate the method of extrapolating values of π/c and $(\eta - 1)/c$ to the value expected at zero concentration, from which molecular weight averages can be calculated. From π/c, number average molecular weight, M_N, is obtained; from $(\eta - 1)/c$ (intrinsic viscosity) a viscosity average, closely related to weight average, M_W, is obtained. Note that π/c at zero concentration is similar for this specimen of dextran and for serum, and so for the dextran-serum mixture, and corresponds to a number average molecular weight of 80,000–86,000.

(Unpublished data, 1954—colloid osmotic pressures—Dr. D. S. Rowe; relative viscosities—Dr. C. R. Ricketts.)

to occur, or in the presence of abnormalities such as oedema accompanying the nephrotic syndrome (see Chapter V). Some quantitative data on the plasma volume expansion actually obtained in patients with various plasma protein levels are given by Metcalf & Rousselot (1953). The plasma volume expansion which they found was generally greater with higher levels of plasma protein.

In Fig. 7*c* and 7*d*, the plots of $\frac{\pi}{C}$ and $\frac{\eta - 1}{C}$ derived from the values shown in Fig. 7*a* and 7*b* respectively are shown, together with the molecular weights which are indicated by the zero intercepts (see p. 17). It will be remembered that the value for $\frac{\pi}{C}$ when C tends to zero gives an indication of *number average* molecular weight, and it will be seen that the dextran specimen tested had a similar *number average* molecular weight to that of the serum proteins. Analogous comparisons between dissimilar substances (dextran, protein) are not valid for the intrinsic viscosity plot shown in Fig. 7*d*, but it is probable that the *weight average* of the dextran is greater than that of the serum proteins owing to its greater dispersion in molecular size.

EXAMINATION OF POSSIBLE SIDE-EFFECTS OF DEXTRAN ADMINISTRATION

The main effects on body constituents of dextran administration have now been outlined. But with any substance intended for widespread clinical use, very careful consideration of possible harmful effects is required. This is particularly necessary when intravenous injection is the rate of administration. Knowledge of the behaviour within the body of all classes of macromolecules is still scanty, and a watch must therefore be maintained for unexpected effects. It is true that carefully conducted animal experiments before administration to patients confirmed the absence of untoward effects. Nevertheless, the wide differences between various animal species and even between individual members of a single species in genetic constitution and in life-history (e.g. previous exposure to micro-organisms) suggests the need for more tests of various kinds. However, as will be shown in Chapter V, administration of dextran to large numbers of patients has resulted in remarkably few reactions. In the rest

of this chapter, tests on animals and in man are described which constitute the evidence so far available for demonstrating possible untoward side-effects of dextran administration.

(*a*) *Antigenicity.* From the time of its introduction as a plasma substitute dextran was claimed to lack antigenicity. The evidence for this claim was the failure to induce the formation of antibodies in rabbits given intravenous injections of relatively large amounts of clinical dextran and the inability to produce active anaphylaxis in guinea pigs (Grönwall & Ingelman, 1944; Bull *et al.*, 1949). These animal tests have subsequently been incorporated in most specifications of clinical dextran solution. On the other hand dextran was known to be precipitated and to fix complement in the presence of certain antisera, and it was therefore regarded as a haptene. Dextran, for example, is precipitated by antisera prepared in rabbits by the injection of suspensions of *Leuconostoc mesenteroides* and other *Leuconostoc spp.* (see p. 44) and, if injected intravenously into guinea pigs, passively sensitized with anti-leuconostoc serum, or with pneumococcal antiserum type II (Hehre & Sugg, 1950) will cause fatal anaphylactic shock.

(i) *Serological Relationships*

The serological properties of dextran have been investigated extensively by Hehre and his collaborators in New York, and it is only possible to mention here certain of the observations which they have made. Hehre (1941), Sugg & Hehre (1942) and Hehre & Neill (1952) showed that precipitin and complement-fixing reactions occur between native and clinical dextrans and certain pneumococcal rabbit antisera, and on the basis of these reactions the native and clinical dextrans were divided into two broad groups:

Group A: precipitate with types II, XII, XX antisera

Group B: precipitate with types II, and XX antisera, and only weakly or not at all with type XII antiserum.

Certain of the dextrans within each of these groups, could be differentiated by their capacity to fix complement with rabbit antisera to *Salmonella typhi*.

The observations of Zozaya (1932*b*) had originally suggested that cross-reactions also occur between dextran and *S. typhi*

anti-serum. Neill & Abrahams (1951) confirmed and extended these observations and showed clearly that native and clinical dextrans gave precipitin and complement-fixing reactions with rabbit antisera to certain members of the Salmonella group (*S. typhi, S. oranienburg, S. paratyphi A.* var *durazzo, S. berta* and *S. cholerae suis*).

The qualitative serological differences exhibited by native dextrans with regard to their reactions with pneumococcal and typhoid antiserum are not lost during the preparation of the corresponding clinical dextrans by hydrolysis (Hehre & Neill, 1952).

Although cross reactions are most commonly observed with pneumococcal antisera types II, XII and XX, Sugg & Hehre (1942) reported two antisera, both type XXII, which gave reactions with native dextran, and Heidelberger & Aisenberg (1953) observed that horse pneumococcal antisera types VII, IX, XI, & XVIII, reacted with two different native dextrans and one partly hydrolyzed dextran.

(ii) *Behaviour as an Antigen*

Evidence has recently been reported that dextran, in its native or clinical form and derived from a number of different strains of *L. mesenteroides*, does behave as an antigen under certain circumstances.

Kabat & Berg (1953) and Maurer (1953) in America have shown that in man the subcutaneous injection of 1.0 mg. of dextran (given as two doses of 0.5 mg.) is frequently followed three weeks later by a rise in serum nitrogen specifically precipitated by the homologous and heterologous dextrans, and by the development of skin sensitivity of the wheal and erythema type to the intracutaneous injection of dextran. Some of the experimental subjects before immunisation were found already to have in their sera measurable amounts of nitrogen, specifically precipitated by dextran, and to exhibit cutaneous sensitivity to dextran. Kabat & Berg immunised groups of volunteers with five native dextrans, three clinical dextrans and one close molecular weight fraction prepared from clinical dextran; the range of the ratio of 1 : 6 to non—1 : 6 linkages represented was from 1.9 to 32 and the range of the number average molecular weights from about 21,000 to several millions. A rise of 2 μg./ml. nitrogen, specific-

ally precipitated by dextran, was considered definite evidence of immunization; a rise of from 1—2 μg./ml. nitrogen was thought to be of possible significance. Using these criteria, immunization was observed in 23 out of 58 volunteers, and occurred in at least one volunteer in each of the nine groups. The sera of 14 volunteers before immunization contained more than 2 μg./ml. nitrogen; of these seven showed a rise of 5 μg./ml. nitrogen after immunization. On the other hand of the 44 individuals whose sera initially contained less than 2 μg./ml. nitrogen, only seven showed an increase of 5 μg./ml. nitrogen or more after immunization. Cutaneous sensitivity was tested before and after immunization in only 24 volunteers. In the four individuals in which an initial cutaneous sensitivity existed, a rise of 2 μg./ml. nitrogen or more occurred after immunization, and was accompanied by an increased cutaneous reaction to the homologous dextran in three; in the fourth no change occurred. Among the ten individuals with a negative or doubtfully positive skin reaction initially, a rise of 2 μg./ml. nitrogen or more, observed in six individuals, was accompanied by the development of a positive skin reaction in four, while in two no significant change occurred.

In the whole group of 58 volunteers, positive skin reactions appeared to be related to the serum level of precipitable nitrogen; 23 out of 24 volunteers with nitrogen levels $< 2\,\mu$g./ml. showed negative reactions while 27 out of 34 with nitrogen levels $> 2\,\mu$g./ml. showed positive reactions.

Too few subjects were examined to allow conclusions to be made regarding the incidence of individuals naturally sensitive to dextran, or the relative antigenicity of the various dextrans, although it was observed that two of the clinical dextrans appeared to evoke greater antibody responses than the other dextrans. It was also observed that the antiserum to the fraction contained two antibodies, one of which appeared to be specific for 1 : 6 linkages and the other for non—1 : 6 linkages. All the other antisera appeared to contain only one type of antibody, specific for the 1 : 6 linkage.

Kabat & Berg considered that the antibodies formed following the subcutaneous injection of dextran were in fact dextran antibodies for the following reasons: (1) the antigenic stimulus was comparable to that needed for immunization to the blood group substances, and only slightly greater than that needed for im-

munization to the pneumococcal polysaccharides; (2) the antibodies reacted with the 17 samples of dextran used (range of ratio of 1 : 6 to non—1 : 6 linkages 1.9—49; number average molecular weight range, 13,000 to several millions) in the *in vitro* tests and gave typical quantitative precipitin curves; (3) if the traces of nitrogen contained in the dextrans used to immunize the volunteers, and not the dextran itself, were the antigen, the ratio antibody nitrogen/antigen nitrogen in the precipitate would be many times higher than has been observed with known protein and other antigens; (4) the lower molecular weight dextrans were more effective per unit weight in precipitating antibody in the region of antibody excess and in inhibiting precipitation in the region of antigen excess.

Kabat *et al.* (1954) using highly purified ^{14}C-labelled clinical dextran and two fractions prepared from this, were able to show that these antibodies specifically precipitated a substantial part of the ^{14}C-labelled dextrans, and concluded that the antibodies formed in man in response to the subcutaneous injection of dextran are indeed antibodies to dextran.

The observations of Maurer (1953), who used some of the same dextrans as Kabat & Berg (1953), as well as other dextrans, to immunize human volunteers, generally confirm those of Kabat & Berg. Maurer also observed that the antibody nitrogen precipitable by dextran persisted with little diminution for many months and that immunization caused little change in the pre-existing level of antibodies to pneumococcal polysaccharides *CI*, *VII*, *SXII* and *SXX*, and absorption of the sera with these polysaccharides caused only insignificant changes in the amounts of nitrogen precipitated by dextran.

The evidence of Kabat & Berg and of Maurer shows, or at least very strongly suggests, that dextran itself under the conditions described, brings about the formation of precipitating antibodies and the development of cutaneous sensitivity to dextran.

Dextran thus appears to behave antigenically in a somewhat similar manner to the pneumococcal polysaccharides and blood group substances, both of which exhibit a species difference. In man the pneumococcal polysaccharides evoke protective antibodies, precipitins and agglutinins and a cutaneous sensitivity of the wheal and erythema type; the rabbit was hitherto thought

not to form antibodies, but Morgan *et al.* (1952) using pneumococcal polysaccharide type II have shown that, if minute doses are given, protective antibodies are formed. The blood group substances are antigenic in man, but not in the rabbit unless they are conjugated with protein. The similarity of behaviour is perhaps further supported by the observation of Glynn *et al.* (1954) that minute amounts of dextran adsorbed on the surface of Group A type IV streptococci, will cause the formation of precipitins and complement-fixing antibodies in the rabbit. Zozaya (1932*a*) had previously shown that nitrogen-free dextran adsorbed on colloidal particles is antigenic in the rabbit.

The antigenicity of dextran in man and the observation that certain normal individuals, who have never received infusions or injections of dextran, may carry antibodies to dextran in their serum and exhibit cutaneous sensitivity, is of obvious importance in relation to the use of dextran as a plasma substitute and to the urticarial and allergic reactions reported in some patients and normal subjects, after the intravenous injection of dextran.

The sensitization of normal individuals may possibly be caused, under suitable conditions, by the absorption of dextran eaten with sugar, of which dextran is a frequent contaminant (Neill *et al.*, 1939), or formed in the nasopharynx by type H streptococci. Hehre & Neill (1946) showed that when strains of this organism, isolated from the throat, were grown in a medium containing sucrose, a polysaccharide was formed which was chemically and serologically similar to dextran; there is no information however whether type H streptococci form dextran in the body. The numbers of volunteers tested by Kabat & Berg and by Maurer are too small to give any indication of the overall incidence of sensitized persons in the American population or to allow conclusions to be made regarding the relative frequency of sensitization to different forms of dextran. It is noteworthy however that Maurer observed significant levels of antibody nitrogen (3—5 μg./ml.), precipitable by the most highly branched dextran he used, in most of the sera of 250 soldiers. The observations of these American authors show that, besides those who are already sensitized and those who react to an injection of dextran by the formation of antibodies, there is apparently a third group of individuals who are non-reactors, but many more individuals

will have to be examined before the relative proportions of these groups can be estimated.

Although small amounts of dextran, injected subcutaneously, give rise to antibodies, the infusion of large volumes of dextran (500 ml. or more of 6 per cent solution) intravenously does not appear to cause sensitization. A series of observations in America on normal individuals (National Research Council, April 1952) showed that second infusions of dextran 4—24 months after the initial infusion were not followed by systemic reactions, except in those who had reacted to the first infusion, and in these, reactions to the second infusions were not more severe than the reactions to the first. Furthermore, the incidence of positive reactions to the intracutaneous injection of dextran in people who had previously been given an infusion of dextran was the same as in persons who had never been given dextran. These observations indicate that the infusion of dextran had not given rise to the sensitivity, and are reminiscent of the findings of Felton & Ottinger (1942), Felton (1949), and Morgan *et al.* (1953) that, while small doses of pneumococcal polysaccaride in mice evoke the formation of antibodies, large doses do not act as antigens but cause a persistent state of "immunological paralysis", during which it is not possible to bring about immunization by injecting small doses of polysaccharide.

(*b*) *Serological detection of the persistence of dextran in the body.* Antibodies to dextran, prepared for clinical use, have not hitherto been produced in the sera of experimental animals, subjected to a series of injections of clinical dextran solution. On the other hand, Evans *et al.* (1941) showed that the intravenous injection of suspensions of *Leuconostoc mesenteroides* in rabbits caused the formation of antibodies which precipitated high dilutions of the homologous highly purified native dextran having a maximum nitrogen content of 0.06 g./100 ml. Similar observations were also made by Hehre (1941) and by Sugg & Hehre (1942). Bull *et al.* (1949) showed that antisera prepared in this way would also give precipitin reactions with high dilutions of clinical dextran solutions; the solutions used by them had a nitrogen content of 0.002 g./100 ml. or less.

Antisera which will precipitate clinical dextran (British, American, Swedish) up to a dilution of 1 in 4×10^6 have been prepared (Maycock, unpublished observations) against *Leuconostoc*

mesenteroides (NCTC No. 2706) *L. dextranicum* (NCTC No. 3.54), *Leuconostoc sp.* known as *Betacoccus arabinosaceous* Birmingham strain (Stacey & Swift, 1948) and *L. mesenteroides* (strain B) from Hehre's laboratory. Certain strains of *L. mesenteroides* appear to be poor antigens in rabbits, e.g. the American strain of *L. mesenteroides*, NRRL–B512. Antisera against these strains of leuconostoc do not give precipitin reactions with normal human or rabbit body fluids (plasma, cerebrospinal fluid, urine, gastric juice, bile), aqueous extracts of the tissue of normal rabbits (liver, kidney, spleen, bone marrow, lymph glands, cardiac muscle, stomach, skeletal muscle, lung, skin, brain and spinal cord), hyaluronic acid, purified preparations of the blood group substances, A, H (human and hog), Lewis (Le^a), or glycogen.

While these antisera precipitate high dilutions of preparations of native and clinical dextrans, none has precipitated oligosaccharides, with a mean chain length less than 17.8 glucose units, derived by acid hydrolysis from dextran synthesized from *Leuconostoc mesenteroides*, strain NRRL–B512. Clinical dextran derived from the latter strain gives a typical precipitin reaction with leuconostoc antisera. Glynn *et al.* (1954) observed that dextrans of molecular weight in the region of 5,000 reacted with dextran antisera (see below) to form specific precipitates. Thus it seems reasonable to assume that the precipitin reactions observed, when body fluids and tissue extracts of animals given dextran are tested with leuconostoc or dextran antisera, are caused by the presence in them of dextran of molecular weight greater than 3,000 to 5,000.

Antisera which will give precipitin and complement-fixing reactions with high dilutions of dextran can also be prepared by immunizing rabbits with a mixture of clinical dextran and type A group IV streptococci (Glynn *et al.*, 1954). Dextrans of molecular weight of about 5,000 were not antigenic under these conditions. Zozaya (1932*a*) immunized rabbits with an antigen composed of nitrogen-free native dextran adsorbed on collodion particles; these antisera precipitated the homologous dextran only in moderately high dilutions. Wallenius (1953) has also described a serological method for the detection of dextran.

The precipitin reaction between rabbit leuconostoc antisera and clinical dextran may be used to study the distribution and per-

sistence of dextran in the tissues of the body, and has the great advantage that it allows the detection of amounts of dextran below the threshold of sensitivity of chemical methods.

The following table (Table III) summarizes the observations made by Bull *et al.* (1949) using the serological method with aqueous rabbit tissue extracts, and includes subsequent unpublished observations made using the same dosage of a closely similar dextran.

TABLE III

PERSISTENCE AND DISTRIBUTION OF DEXTRAN IN RABBIT TISSUES

Total intravenous dose: 9 g. dextran/kg. body weight given as 6% clinical dextran solution over 7 days.
Dextran detected by serological method.

	2 *months*[1]	6 *months*[2]	9 *months*[2]	12 *months*[2]
Liver	+	+	+	trace
Kidney	+	+	+	trace
Lymph glands	+	+	+	trace
Spleen	+	+	+	0
Bone marrow	–	+	+	0
Skeletal muscle	–	+	0	0
Cardiac muscle	–	+	0	0
Lung	–	+	0	0
Stomach	–	+	0	0
Brain	–	+	0	0
Spinal cord	–	+	0	0

(– = not tested)

[1] Data from Bull *et al.* (1949).
[2] Data from Maycock (unpublished observations).

After an intravenous dose of 1.2 g. dextran per kg. body weight in rabbits (given as 6 per cent clinical dextran solution), which is approximately equivalent to an infusion of 1,400 ml. in a man weighing 70 kg., the distribution and persistence of dextran follow the same pattern as that shown in Table III. Four months after injection dextran is detectable in the liver, but in no other tissues; six months after injection dextran cannot be detected in any of the tissues shown in Table III. It was also observed

that the cerebrospinal fluid gave a positive precipitin reaction four weeks after injection, and that the excretion of dextran in the urine can be detected serologically for about three months, although dextran is no longer detectable in the serum after about twenty-four days. The amounts excreted in the urine after the initial outflow in the first 24—48 hours after injection are small (cf. p. 25) and account for only a very small part of the total dose. This dextran is probably derived from the larger molecules of dextran which have been retained in the tissues and gradually broken down to a size which can traverse the glomerular capsule. Their concentration in the serum is below the threshold of the serological method, but is increased sufficiently during the passage of the dextran through the kidney to bring it within detectable limits (Lorenz & Maycock, unpublished observations).

Two and a half years after a series of injections of 6 per cent dextran solution equivalent to 84 g. dextran, dextran could not be detected serologically in aqueous extracts of the rabbit's tissues (Lorenz, 1954).

Traces of dextran have also been detected in the gastric juice and bile of patients and of rabbits for several days after the injection of dextran solution.

Closely comparable results are observed in the mouse. After an intravenous injection of 60 mg. dextran, Lorenz (1954) observed the excretion of dextran in the urine over a period of nearly three months, and found that traces of dextran were still present in extracts of whole mice after eight months. Weight for weight, an intravenous injection of 60 mg. dextran in a mouse weighing 20 g. is equivalent to an infusion of 3.5 litres of 6 per cent dextran in a man weighing 70 kg.

The observations made by means of the serological method show that:

(1) Injected dextran, although widely dispersed in the tissues of the rabbit, is found mainly in the liver and other tissues rich in reticulo-endothelial cells, such as the lymph glands, spleen and bone-marrow.

(2) Dextran gradually disappears from all tissues, remaining longest in liver and other tissues rich in reticulo-endothelial cells, and its persistence appears to be proportional to the dose given.

(3) The excretion of dextran in the urine continues for long periods at a concentration below the sensitivity of chemical methods of detection.

(4) Dextran appears in the gastic juice, bile and cerebrospinal fluid.

The observations of Persson (1952*b*) and of Mowry & Millican (1953) suggest that much of the dextran detected in the various tissue extracts examined by the serological method was contained *in vivo* in cells of the reticulo-endothelial system. The precipitin reaction is a more sensitive and more specific means of demonstrating the presence of dextran than the available chemical or histochemical methods.

(*c*) *Histochemical Demonstration of dextran in tissues.* The glycol groupings present in many polysaccharides are oxidized by periodic acids to aldehyde, which will react with leucofuchsin (Schiff's reagent) causing it to become coloured. These reactions have been made the basis of methods for staining a wide range of polysaccharide (McManus, 1946; Hotchkiss, 1948) and have been used by Friberg *et al.* (1951, 1953), Persson (1952 *a* & *b*), Mowry *et al.* (1952) and Mowry & Millican (1953) to study the distribution of dextran in the tissues of mice, guinea-pigs and rabbits after intracutaneous, subcutaneous, intravenous and intraperitoneal injection.

Chromic acid and potassium permanganate are also used as oxidizing agents in combination with Schiff's reagent for staining polysaccharides; the reactions involved are less well-understood, but are thought to involve the glycol groupings. Persson (1952*a*) states that both these methods gave positive reactions with glycogen and negative reactions with dextran although he observed a positive reaction on one occasion after oxidation of dextran with chromic acid, and that a substance, presumed to be dextran, in certain granulocytes gave a positive reaction to Schiff's reagent after treatment with potassium permanganate.

After oxidation with periodic acid, both glycogen and dextran are said to stain with basic dyes, such as toluidine blue, Azure A and basic fuchsin; after oxidation with chromic acid or potassium permanganate, dextran, unlike glycogen, stains poorly or not at all (Persson 1952*a*). According to Persson (1952*b*) dextran takes on a pale yellow colour when stained with Gram's iodine, in contrast to the reddish-brown staining of glycogen.

Since dextran is readily soluble in water, tissues containing dextran must not be brought into contact with watery reagents. The application of these staining reactions to the identification of dextran in tissues has been most fully studied by Persson (1952*b*) and by Mowry & Millican (1953) whose papers should be consulted for details of the methods used, none of which is claimed to be specific for dextran. Persson (1952*b*) used a combination of methods and regarded as dextran only that material in the tissues of animals given dextran, which met the following conditions: (*a*) gave a strong positive periodic acid Schiff reaction (*b*) gave a negative chromic acid Schiff reaction (*c*) gave a strong bluish violet colour with periodic acid Azure A (*d*) gave no metachromasia with Azure A (*e*) took a pale yellow colour with Gram's iodine. As Persson states, it is not possible always to be certain that a substance meeting these conditions is indeed dextran, but the presence of dextran may be presumed with some certainty, if due weight is given to the presence and distribution of Schiff-reactive substances in control tissues from uninjected animals.

Mowry & Millican (1953) regarded as dextran any substance (in the tissues of mice, previously given dextran) which was stained by the alcoholic periodic acid aqueous Schiff's method and which was dissolved out of the tissues when aqueous oxidising reagents were used. With the exception of gland-neck mucin of the stomach, they found no other material which met their staining requirements in the tissues of mice to which dextran had not been given. Both Persson and Mowry & Millican starved most of their animals to deplete the reserves of glycogen, which otherwise rendered the interpretation of the staining reactions more difficult in certain tissues.

The amount (and possibly the pattern of distribution) of dextran found in the tissues will depend upon the dose injected and its molecular size. The findings of Persson and of Mowry & Millican in rabbits and mice injected with Swedish and American clinical solutions are similar. Immediately following injection, granular material, fulfilling the staining criteria adopted by these authors and therefore assumed to be dextran, was found within the blood vessels lying extracellularly and within granulocytes, and extracellularly in the cortical and medullary sinuses of the lymph nodes. Subsequently, the amount gradually diminished. In the kidneys

dextran appeared within 15 minutes in the subcapsular spaces and in the lumina of the nephrons. After an hour or so it was found in the epithelium of the proximal convoluted tubules and in the cells lining the distal parts of the nephron; deposition reached a maximum after a few hours after which the amount gradually diminished. Dextran persisted in macrophages in the renal interstitial tissue long after it had gone from the parenchymal cells. In the liver, dextran appeared in the parenchyma and Kupffer cells approximately at the same time. The deposits reached a peak several hours after injection and then disappeared relatively quickly from the liver cells. The Kupffer cells, however, like the reticulo-endothelial cells of other organs, retained dextran for long periods of time. Initially, the spleen showed large numbers of granulocytes containing dextran; these gradually diminished in number, and the dextran was then found after 2—3 days in free and fixed macrophages in the red pulp. Similarly dextran was observed in free and fixed macrophages in the lymph nodes, the bone marrow, lungs, stomach, small intestine, pancreas, ovary, adrenal, thyroid and thymus glands, myocardium, fat and skin. Dextran was also sometimes detected in the cells of the zona glomerulosa of the adrenal medulla. The rate of disappearance of dextran from the reticulo-endothelial system was directly proportional to the dose injected into the animal. Mowry & Millican's observations led them to suggest that in certain reticulo-endothelial cells, e.g. Kupffer cells, the amount of dextran gradually declined with the passage of time, while in the other tissues, the number of dextran-laden macrophages, rather than the amount of dextran within each cell, appeared to diminish.

Persson (1952*b*) confirmed the observation of Friberg *et al.* (1951), that dextran appears to exert a positive chemotactic effect on polymorphonuclear leucocytes and observed that the staining properties of the (presumed) dextran granules within these cells were different from those of similar granules in other cells. In leucocytes these granules gave a positive Schiff reaction after oxidation with periodic acid or potassium permanganate, and a negative iodine reaction, thus behaving neither as glycogen, nor as dextran in other tissues. Although it is known that the polymorphonuclear leucocytes normally contain Schiff positive granules (Wislocki & Rheingold, 1949), leucocytes giving this reaction increased greatly in number after the injection of dextran,

and Persson considered it probable that these cells were carrying dextran, possibly in a modified form.

The histochemical observations of Persson, Friberg *et al.* and Mowry & Millican agree broadly with earlier findings of Bull *et al.* (1949) and of Lorenz & Maycock (unpublished observations) who, using a serological technique for the detection of dextran in tissue extracts, found that the dextran which is retained in the body after intravenous injection, is widely distributed but occurs predominantly in those organs rich in reticulo-endothelial cells and that it gradually disappears (see p. 46). The histochemical technique in its present form suffers from the serious disadvantage that it is not specific. None of the histochemical and empirical staining techniques is capable of distinguishing dextran from glycogen and mucopolysaccharides by positive methods, and when dextran concentration in the tissues falls below that of these naturally-occurring substances identification becomes extremely difficult, and is largely a matter of personal judgment. It cannot therefore be regarded as being as sensitive as the serological technique which appears to be specific. A combination of the serological and histological techniques by the use of fluorescent antibody (Coons & Kaplan, 1950) should, theoretically, provide a sensitive and specific means of identifying dextran in tissue sections.

(*d*) *Histological changes after the administration of dextran.* The kidneys and liver of animals which have been given solutions of native dextran intravenously show necrotic foci, which are probably secondary to thrombosis of the capillaries (Grönwall & Ingelman, 1945).

Most authors report that clinical dextran solution, when given to normal dogs, rabbits and mice, even in volumes equivalent to 20.0 g. dextran/kg. body weight over one month, does not cause histological abnormalities (Grönwall & Ingelman, 1945; Ingelman, 1947; Bull *et al.*, 1949; Thorsen, 1949; Friberg *et al.*, 1951; Persson, 1952*a*; Mowry & Millican, 1953), and the opinion generally held at the present time is that modern clinical dextran solutions do not act as tissue irritants when given in moderate amounts.

There are, however, a few records of histological changes in various organs attributed to the intravenous administration of clinical dextran solution, but in most of these experiments very

large doses of dextran have been given. Goldenberg *et al.* (1947), who used a solution prepared in U.S.A. from imported dried Swedish dextran, found pronounced but transient changes in the renal tubules of rabbits given 40 g. dextran/kg. body weight over some 16 weeks. Renal function was unimpaired. Turner *et al.* (1949) reported focal degenerative changes in the liver and kidneys and slight reticulo-endothelial hyperplasia in the spleens of dogs, sacrificed between the 5th and 19th days after bleeding and the infusion of from 400 to 500 ml. of Swedish dextran solution. Hartman (1951), who administered an American clinical dextran solution to mice in an amount equivalent to 12.6 g./kg. over three weeks, observed swelling, vacuolation and desquamation of the epithelium of the proximal convoluted tubules, infiltration by foam cells and occasional rupture of the blood vessel walls, especially in the lungs, and many foam cells in the lymph nodes. Most of these changes were temporary. Nelson & Lusky (1951) state that only very minor changes occurred in rabbits given 9.6 g./kg. Swedish dextran over two months.

Friberg *et al.* (1953), using rabbits, administered clinical dextran solution equivalent to about 75 g. dextran/kg. body weight over 3½ to 4 months. No pathological changes were observed except in the lungs and spleen. In the lungs the peribronchial connective tissue contained an increased number of lymph follicles. Multinucleate giant cells were scattered through the red pulp of the spleen; as these did not apparently contain dextran, Friberg *et al.* suggest that their occurrence was probably to be interpreted as a reaction to the dextran. Unfortunately, Friberg *et al.* only examined animals immediately after the course of injection of dextran, so that there is no evidence whether these changes, like those observed by Hartman (1951), were temporary.

(*e*) *The reaction of the rat to dextran.* Dextran can be given to most laboratory animals without causing reactions. The intravenous or intraperitoneal administration of dextran (300 mg./kg.) to rats, however, is followed in a few minutes by the appearance of stupor, dyspnoea, congestion of the paws and snout, and frequently scratching. Some 15 or so minutes later oedema of the paws and snout develops and subsides in about two hours (Vorhees *et al.*, 1951, Morrison *et al.*, 1951). Diarrhoea, sometimes bloody, may follow, and during recovery, the animals may show signs of extreme thirst.

This syndrome is similar to that observed in rats after the injection of egg albumin (Selye, 1937; Halpern & Briot, 1950). Morrison and his collaborators observed that pretreatment with the antihistamine drug, pheniadamine, or with cortisone, would prevent the formation of oedema in almost all their animals and that procaine and procaine amide would protect some animals. The reaction appears to be caused by a local increase in capillary permeability. Edlund *et al.* (1952) using Menkin's intravenous dye test observed seepage of dye into wheals raised by the intradermal injection of dextran dissolved in 0.9 per cent saline solution, providing the concentration of dextran was at least 10 μg./ml. These authors also report that highly branched dextrans cause this reaction more readily than less branched dextrans, and that small molecular dextran is less effective than larger molecular dextran. Edlund *et al.* found that alloxan, given intravenously in sub-diabetogenic doses, was the most effective inhibitor, and that BAL (2, 3-dimercaptopropanol) was effective only in some animals. Halpern & Briot (1952) found that histamine was liberated *in vitro* by freshly excised pieces of rat skin at 37°C, but not at 0°C, in the presence of dextran.

The reactions observed in the rat have not been seen in any of the other laboratory animals so far used in investigating dextran and appear to be peculiar to this animal. They are probably unrelated to the reactions observed in man (see p. 67).

(*f*) *Haemostatic defect after infusion of dextran in normal subjects.* Carbone *et al.* (1954) have observed that the bleeding time can be prolonged in normal individuals if sufficiently large volumes of dextran solution are given. The volume of solution required to cause prolongation of the bleeding time varied from 1000 ml. to 6,500 ml. (not more than 1500 ml. were given in one day). The results of their investigations seem to show that this phenomenon cannot be explained by any defect in the clotting mechanism itself, since none of the changes observed (prothrombin, activity, prothrombin consumption, clotting time, clot retraction, factors V & VI, anti-thrombin titres) were sufficiently great. Further, the effect is probably unrelated to changes in plasma volume, since the maximal increase in volume occurred immediately after infusion and the maximum prolongation of bleeding time appeared some three to nine hours after the end of the infusion. The bleeding time returned to normal within 24 hours.

The cause of this abnormality is not known. It is possible that the tendency to haematoma-formation mentioned by Boyd *et al.* (1953) is attributable to this haemostatic defect.

(*g*) *Infection-promoting activity of certain polysaccharides.* Certain polysaccharides are able to promote infections. A crude preparation of gastric mucin, for example, will reduce the minimum lethal dose of bacteria injected intraperitoneally if given at the same time as the bacteria (Nungester *et al.*, 1932). Shilo *et al.* (1953) and Hestrin *et al.* (1954), who investigated the infection-promoting activity of dextran and levans, found that native dextrans, given either intraperitoneally or intravenously to mice at the same time as one-tenth M.L.D. of a suspension of *Salmonella typhi* 0—901 administered intraperitoneally, strongly promoted infection, 70 per cent of the mice dying. Less than 5 per cent of the mice, which received only the bacterial suspension, died. The reduction of the molecular size of dextran and levan by partial hydrolysis and fractionation diminished their ability to promote infection. Dextran, of a molecular weight in the range 10^4 to 10^6, prepared by Shilo and his collaborators, and one commercially prepared clinical solution of dextran exhibited negligible infection-promoting activity.

As far as is known, no clinical reports have appeared which suggest that dextran solution enhances or facilitates infection in humans. Whether any practical significance is to be attributed to the observations of Shilo and his colleagues must await further clinical evidence and laboratory investigation.

CHAPTER IV

SPECIFICATION OF DEXTRAN

THE purpose of a specification for a therapeutic substance is to ensure that a consistently effective material is available for clinical purposes. Variations in response may then be attributed solely to the patient's condition and the knowledge of the best way of using the remedy becomes cumulative. Such a specification should be modified in the light of clinical and laboratory experience. Broadly speaking one requires that dextran should be effective in reproducible fashion, and harmless.

Specification of dextran, which may be regarded as a biologically produced high polymer, presents novel difficulties. No simple test of efficacy is available. Testing in man may be in volunteers or in series of patients, preferably with suitable controls. The response of the blood volume of normal volunteers to injections of dextran has been investigated but this is not a good parallel to the clinical use of the material. A better test is to infuse the material after the subject has had his circulatory volume reduced by bleeding (cf. Chapter III). Such procedures are useful research methods but are not feasible for routine purposes. Similar criticisms apply to tests on patients where the method might be to treat parallel controlled series of patients with similar injuries comparing the effects of dextran with a standard transfusion material such as plasma. In animals no satisfactory simple test of efficacy for routine use has been devised though one may be found in the future. The nearest approach has been the animal excretion test described later, which is a check on the loss of material through the kidneys and hence on the amount potentially remaining available for maintenance of circulatory volume.

Tests for sterility, pyrogenicity and toxicity follow the regulations in force in the various countries and will not be discussed here. Tests for antigenicity are open to debate in the light of the findings of Kabat & Berg (1953) and the reader is referred to page 40.

MOLECULAR COMPOSITION

Since it is widely agreed that efficacy is related to molecular composition this is always specified, and various tests are used. These tests are intended to ensure that the molecular weight distribution is similar to that of dextran batches which have been clinically tested and found effective in maintaining plasma volume. They must provide a check both of average molecular size and of the proportions of large and small molecules. Large molecules are considered undesirable in view of early reports of tissue damage and because of their interference with the cross-matching of blood. Small molecules are rapidly excreted by the kidney and lost into tissue fluids and while they may or may not cause undue diuretic effects or produce excessively viscous urine, they are certainly wasteful of therapeutic effect.

The optimum size of dextran molecules is a matter of debate. There is general agreement that dextran should be retained in the circulation for the period during which an expansion of plasma volume is desired; but while some clinicians are content with a temporary effect such as might be achieved with small molecular material, others demand that an adequate plasma substitute must be able to stay in the circulation until natural replacement of plasma protein occurs.

CHEMICAL AND PHYSICAL SPECIFICATIONS FOR CLINICAL DEXTRAN

Specifications for clinical dextran have been published in the U.S.A. and Britain. Those parts of the specifications referring to chemical and physical properties are shown in Table IV. Tests for sterility, pyrogenicity and antigenicity are included in the British and American specifications; the latter also includes a mouse test for toxicity which has been omitted from the British specification since 1952 (cf. page 60).

These figures together with the comparative data in Table I show that prevalent opinion in the U.S.A. has been in favour of a dextran of lower average molecular weight and consequently higher renal excretion than that currently accepted in Britain. However, it should be emphasized that all shades of opinion are to be found in both countries and the specifications quoted are likely to be modified in the light of ever increasing clinical experience by which all preparations must ultimately be judged.

On this basis it seems probable that opinions will tend to converge.

TABLE IV

CHEMICAL AND PHYSICAL SPECIFICATIONS FOR CLINICAL DEXTRAN

U.S.A. (Military Medical Purchase Description, 1954)	*Great Britain* (Ministry of Health Specification, 1954)
Analysis:	*Analysis*:
Dextran 5.7–6.3 g./100 ml.	Dextran 5.5–6.5 g./100 ml.
Sodium chloride 0.85–0.95 g./100 ml.	Sodium chloride 0.85–0.95 g./100 ml.
Buffering capacity $\geq$ 3.0 ml. 0.1 N NaOH/litre	Potassium $\geq$ 25 mg./100 ml.
pH 4.5–7.0	pH 5.0–7.0
Nitrogen < 1.00 mg./100 ml.	Reducing sugars < 100 mg./100 ml.
Heavy metals as lead < 0.5 mg./100 ml.	Acetone < 0.02 g./100 ml.
Ash (less NaCl) < 0.05 g./100 ml.	Nitrogen < 1.00 mg./100 ml.
	Heavy metals as lead < 0.5 mg./100 ml.
Molecular Composition:	*Molecular Composition*:
Whole polymer M_W 75,000 ± 15,000	Intrinsic viscosity 0.32 ± 0.03 dl./g. in aqueous solution at 37°C.
High 10% fraction M_W $\geq$ 200,000	High 10% fraction—intrinsic viscosity $\geq$ 0.53 dl./g. in aqueous solution at 37°C.
Low 10% fraction M_W $\leq$ 25,000	Renal excretion (under stated conditions in rabbits) < 25%
Inherent viscosity 0.255 ±0.035 dl/g. in aqueous solution at 25°C.	

A few comments on the tests required by these specifications may be useful. Difficulties in determining the concentration of aqueous dextran solutions have been referred to on page 20. Estimates of concentration by the various methods are sufficiently reproducible for most purposes but the difficulty of deciding whether a dextran sample dried to constant weight is in fact free from moisture introduces an uncertainty which may be of the order of ± 0.3 per cent. Nitrogen estimation is difficult since dextran must be oxidized to carbon dioxide to leave less than

1mg. nitrogen as ammonium sulphate in the Kjeldahl method. Potassium concentration was limited in the British specification to the normal plasma level, not because any could be found in clinical dextran solutions, but because of the view that an excess of potassium ions may adversely affect patients in a state of shock. Since most dextrans are fractionally precipitated some limit on the precipitating solvent, e.g., acetone, seems advisable. Reducing sugars would be included as dextran by many methods of dextran determination and their concentration is therefore limited in the specification.

As to the main feature, molecular composition, it will be seen that the specifications are strikingly different in the forms of test adopted. The British specification, originally written in the autumn of 1948 on the basis of the early clinical trials (Bull *et al.*, 1949) stood the test of practical experience for three years with the results summarized by Maycock (1952). Since then some batches with intrinsic viscosities as high as 0.37 have been used without mishap. In 1948 it was felt that intrinsic viscosity was a sufficiently reliable, albeit empirical, measurement of molecular weight, and that the renal excretion of dextran by rabbits provided a realistic estimate of the excretion to be expected in patients. As the intrinsic viscosity measurement is especially affected by the larger molecules present while the excretion test provides a check on the smaller molecules, a reasonably satisfactory limitation of molecular weight range appears to be effected by a combination of the two tests. Furthermore as improved production methods lead to a smaller proportion of excretable molecules, adherence to a similar average size as determined by intrinsic viscosity would automatically require a preparation with a narrower dispersion.

Although it is very difficult to discover detailed evidence of bodily harm attributable to the large molecules, it is strongly felt that some limit on their presence should be maintained. An arbitrarily chosen limit of intrinsic viscosity 0.53 for the upper 10 per cent fraction has been accepted since 1952 as an addition to the earlier British specification.

Since 1948 knowledge of the scattering of light by colloidal particles has advanced considerably and the commercial production of instruments for this measurement has enabled a more precise form of specification to be adopted. However

it should be pointed out that though physico-chemical tests are admirably suited to maintaining a constant quality they must be run in parallel with biological tests at least long enough for their significance in biological terms to emerge. This is especially to be emphasized in the case of a preparation such as dextran, which in fact comprises a group of substances varying in detailed structure (see for example Fig. 4) so that the preparation offered for clinical use may vary from time to time.

When specifications demand the preparation of 10 per cent fractions ("tops" and "tails") it must be appreciated that the more carelessly these fractions are prepared the more likely they are to pass a test defining molecular weight through being more heavily contaminated with molecules of medium size. This of course is the reverse of what is desirable in a specification, and such a test can only be accepted if details of preparing the "tops" and "tails" are carefully laid down.

VALUE OF BIOLOGICAL TESTING

Although physico-chemical specification offers advantages over biological testing in economy and precision in certain directions, there is much to be said for retaining biological criteria for the control of some aspects. Dextran is not a simple molecular species; small changes in structure resulting from minor alterations in the manufacturing process or from minor variations in the parent strain or organism or in the medium could conceivably alter the biological properties of the final product. The renal excretion of dextran in animals, which is closely related to the properties of the dextran below a critical molecular size, should be measured. A physico-chemical specification, which limits the proportion of dextran of a molecular weight below 25,000 and defines the mean molecular weight of the whole preparation as, for example, the U.S.A. specification, only loosely controls the proportion of dextran of molecular weight between 25,000 and 45,000 to 50,000, which is approximately the renal threshold. A limit of dextran excretable by an animal in a given time would appear to control this aspect more rigidly than a physico-chemical specification. Testing for antigenicity by attempting to evoke precipitins in rabbits by the injection of dextran was formerly a required test in the British specification but has been discontinued since over several years no batch was rejected on the basis of this

test. Similarly the test for toxicity in mice (1.0 ml. intravenously) was discontinued for the same reason and also because it was considered that the intravenous injection of dextran in the pyrogen test was as likely to disclose harmful effects. On the other hand a test for anaphylaxis in the guinea-pig has much to recommend it and it would seem wise to retain it until the basis of the reactions observed in humans has been elucidated. Future work may show how best to determine the possible differences between antigenic properties of different dextrans. Meanwhile it is important to remember, especially if new types of dextran are to be recommended, that biological behaviour is our closest laboratory measure of therapeutic efficacy.

FUTURE IMPROVEMENTS

As the opportunity for varying the specification of dextran arises in future it seems desirable to include a statement of the branching ratio of the dextran as determined by periodate oxidation. Since there are still unknown features in the structure of dextran molecules, viz., whether the branches are long or short, the strain of leuconostoc producing the dextran should in any case be stated. Dextran is valued for one fundamental physiological property, namely its osmotic pressure in admixture with human plasma. A statement of the dextran concentration required to give the physiological colloid osmotic pressure (i.e., 40 cms. of water) under definite conditions of mixture with human serum *in vitro* (Rowe, 1954) might come near to a statement of potency. When practical progress makes routine measurements technically feasible, osmotic pressure estimates of number average molecular weight (M_N) and light scattering estimates of weight average molecular weight (M_W) should be included, and replace intrinsic viscosity measurements. We look forward to the development of a clinical dextran free from molecules so small as to be rapidly lost from the circulation and from appreciable amounts of very large molecules. International agreement on satisfactory standards for this therapeutic substance is desirable.

CHAPTER V

CLINICAL USE OF DEXTRAN

IN STATES OF "SHOCK"

RESTORATION of the circulatory volume is the most important single measure in the treatment of severely injured patients. When whole blood has been lost, replacement by transfusion of blood is the treatment of choice, but where the loss is largely of plasma or serum it is rational to use a colloid solution such as dextran. Even when blood has been lost, the circulatory volume can be satisfactorily corrected by dextran, as has been demonstrated in numerous animal and human experiments (Grönwall & Ingelman, 1945; Bull *et al.*, 1949; Turner *et al.*, 1949; Bollman *et al.*, 1951; Wilson *et al.*, 1952; Hammarsten *et al.*, 1953; Wasserman & Mayerson, 1954). Since no red cells are replaced a dilution of the remaining cells results and a temporary anaemia is caused, which is better avoided if blood is available. From experimental studies on dogs subjected to severe haemorrhage, Parkins *et al.* (1953) concluded that dextran and other plasma substitutes, though much superior to equal volumes of saline, were inferior to whole blood for purposes of resuscitation.

Many of the studies of the clinical results of circulatory volume replacement with dextran concern its use during severe surgical operations and for post-operative haemorrhage. The early reports from Sweden (Bohmansson *et al.*, 1946) gave details of several such cases and showed that blood pressure was readily restored to normal values. Similar satisfactory results were obtained in the British trials (Bull *et al.*, 1949; Boyd *et al.*, 1953), and it was confirmed that whereas dextran raised and maintained the blood pressure in conditions of shock, saline solution alone was by comparison ineffective. American surgeons using both Swedish and American dextran also reported favourably on its value for operation cases (Craig *et al.*, 1951; Spence *et al.*, 1952).

In the treatment of shock from severe accidental injuries associated with blood loss, most workers who use dextran prefer to supplement it with blood transfusion. Wilkinson (1951), who

reported results in a group of severely injured patients treated with Swedish dextran, confirmed that good effects were often obtained after rather rapid infusion and he gives details of some cases in which blood pressure was restored surprisingly promptly. Since it is known that dextran can increase circulatory volume initially by an amount greater than the volume administered it seems likely that this effect which has also been noted by others is due to the dextran being somewhat hyperoncotic and therefore attracting water into the circulation. American workers have also obtained good results in the treatment of wound shock but emphasize that for the most severe injuries blood should be given in addition to dextran. Haynes and DeBakey (1952), reporting the results of treatment of 20 cases on which circulatory volume studies were also made, suggest that a reasonable maximum severity for treatment with dextran alone should be blood loss of 35 per cent. A haemorrhage of 2 litres or more in an adult such as commonly results in a fall of blood pressure to 70 mm. or less should on this criterion be treated with whole blood transfusion as well as dextran. These authors conclude that "depending on the complexity of the clinical problem and the percentage of blood volume lost, dextran may provide sufficient circulatory support to permit reparative surgery or minimize the quantity of whole blood necessary to accomplish this aim".

Dextran has also been successfully used in the treatment of patients who have lost fluid predominantly into the peritoneal cavity and alimentary canal. Bohmansson *et al.* (1946) and Wilkinson (1951) report success in cases of acute diffuse peritonitis and ileus using Swedish dextran. It is not easy to assess how much fluid is lost in such conditions, but the restoration of blood pressure and clinical improvement suggest that a colloid solution such as dextran has a valuable role in treatment. It is likely that dextran will be useful in the treatment of several other forms of "shock". Experimental crush injury in dogs has been found to respond well (Grönwall & Ingelman, 1945). It is also probable that certain toxaemias may benefit from maintenance of circulatory volume; Pedersen & Christensen (1952) report successful treatment of six cases of botulism for which transfusion with dextran and blood was thought to be of value.

Burns are a common type of injury in which loss of plasma predominates over loss of red cells and some of the earliest cases

successfully treated with dextran were of this type. Rosenkvist (1947) in a careful clinical study of treatment of burns gives details of the transfusion of 47 burns shock cases. Many of these patients received plasma or blood as well as dextran and it was the author's opinion that results with dextran were as good as those with plasma in the treatment of burns. His success with two large burns cases treated with dextran and blood led to a subsequent more detailed study (Rosenkvist & Thorsen, 1951) which showed that good results could be obtained when dextran and blood were given alternately to burns patients. The ten cases reported received two or three times more dextran than blood and included a number of very favourable results. Johnston *et al.* (1953) also using Swedish dextran, obtained good results in a small series of patients with burns. They recommended that plasma or whole blood also should be given for the more severe cases. The early trials with British dextran (Pain *et al.*, 1949) included detailed studies of two patients with burns treated entirely with dextran. One, a moderately large burn in a child, did well; the other patient, an adult with very severe burns, died, though no ill effects of dextran were found. Subsequently, British dextran has been used on a series of 100 burns shock patients (Bull & Jackson, 1955), and though good results were frequently obtained with dextran alone, in some severely burned patients the large amounts of dextran which needed to be transfused resulted in very low plasma protein levels. Some of these patients did not seem subsequently to recover as well as might have been expected, and the procedure was adopted of limiting the amount of dextran to be transfused to any single patient to a volume equal to the approximate plasma volume of the patient. Consequently, after treating the first 21 patients by giving the necessary amounts of colloid as dextran without limit, 79 patients were treated on this regime of limited volume, continuing the transfusion with plasma or blood if more colloid was required. The results on these cases were clinically equal to those in which plasma and blood only had been used. Table V gives a summary of all these cases and shows the comparison between the observed mortality together with that "expected" on standard treatment with plasma and blood as calculated from the statistical study of Bull & Fisher (1954). It will be seen that whereas the earlier 21 cases receiving dextran only, did not have such a

TABLE V

100 BURNS SHOCK CASES TREATED WITH DEXTRAN (1950–3)

(Data of Bull & Jackson, 1955)

Dextran	*No. of cases*	*Area of Burn*		*Mean quantities transfused (ml.)*				*Deaths*	*"Expected" Deaths*
		Mean	*Range*	*Dextran*	*Plasma*	*Blood*	*Total*		
Volume not limited	21	20%	10—47%	1775	390	182	2347	3	1.2
Volume limited to about one plasma-volume and supplemented with plasma or blood when necessary	79	25%	10–80%	851	829	457	2137	16	15.5
	100	23%						19	16.7

favourable outcome as the average expected, those treated with the limited volume of dextran had as good a mortality result as cases receiving standard treatment. It will be noted that in each series the mean total colloid solution transfused was about 2.3 litres. It appears that the use of dextran in quantities up to one plasma volume resulted in an economy of some 67 litres of plasma or blood in the treatment of these 79 patients suffering from severe burns.

It is clear from the numerous reports coming from many countries that dextran is effective as a plasma volume expander in states of shock, and that it has given clinically satisfactory results in many thousands of transfusions. Its osmotic effectiveness should always be borne in mind when dosage is being considered so that excessive quantities are avoided. Administration of dextran to patients with an already normal blood volume or in marked over-correction of a loss can readily cause overloading of the circulation and raised venous pressure.

FOR RELIEF OF OEDEMA IN THE NEPHROTIC SYNDROME

Success in relieving oedema by infusing dextran to patients with the nephrotic syndrome, both children and adults, has been reported from Sweden (Wallenius, 1950), the United States (Olive *et al.*, 1953, James *et al.*, 1954) and Britain (Mollison & Rennie, 1954). Detailed case reports are given by these authors. Wallenius obtained diuresis in each of 4 patients tested, Olive obtained significant diuresis in 7 out of 12 patients, and Mollison and Rennie reported satisfactory results in 6 out of 7 patients. James *et al.* noted significant loss of oedema in 9 out of 13 children, and virtually complete diuresis in 6 of these patients. Bedford & Broughton (1951) record failure to induce diuresis with two preparations of dextran in a patient with a grossly raised blood urea, affected by nephrotic oedema.

From the practical point of view, several points emerge from these studies. Dextran solutions must be given slowly (e.g., 30 g. to an adult in not less than 4 hours) and not in excessive amounts (e.g. 15—30 g. to a child, 30—80 g. to an adult). In the presence of oedema with hypoproteinaemia, considerable expansion of plasma volume always occurs (e.g. by 60 per cent following 30 g. infused), as evidenced by a marked fall in haematocrit and in plasma protein level. Although the initial

plasma volume of these patients is often 20—30 per cent below normal, such rapid increases are often accompanied by a sense of fulness or pain in the head, and by slight engorgement of the neck veins (Squire, personal observations). Death from pulmonary oedema or congestive cardiac failure could almost certainly be caused by overdosage in these circumstances. James *et al.* (1954) found elevation of systolic and occasionally diastolic pressures during infusions. Headache, nausea and abdominal pain sometimes occurred. Epistasis and haematuria were also noted.

Usually, the diuresis following single infusions to such patients is transient, lasting only 24—48 hours. An increased loss of sodium and chloride also follows infusion so that oedema fluid is excreted but the effective removal of much oedema necessitates repeated infusions every few days. Exceptions to this rule occur (Squire, 1953) as with other colloidal infusions such as gum acacia, previously given for relief of oedema; when the rapid relief of oedema follows only one or two infusions, it may be suspected that spontaneous diuresis might soon have occurred in the absence of intravenous therapy. Olive *et al.* (1953) specifically note their disappointment with dextran as a form of treatment, in that recurrence of oedema at the end of a course of treatment was the rule. Wallenius, on the other hand, found that when oedema was relieved by 7 or 8 infusions, the appetite of his patients improved and relief lasting at any rate for months might follow. In brief, this form of therapy does not seem to lead to any radical change for better or for worse in the behaviour of the diseased kidney. After disappearance of dextran from the blood and return of plasma proteins to pre-infusion level, proteinuria is neither less nor more than before infusion. As high protein diets appear slowly to improve these patients, a period of symptomatic relief enabling a better dietary intake to be instituted may help the patients indirectly.

Nevertheless, studies of this kind have helped to throw light on the puzzling group of conditions classed as the "nephrotic syndrome". The sudden rise in sodium chloride excretion following an infusion which apparently mainly effects an increase in plasma volume—either with salt-free dextran which depresses plasma protein level or with salt-free albumin which causes a transient rise—proves that these diseased kidneys are by no means incapable of excreting chlorides under the appropriate physio-

logical stimulus. Another finding yet to be explained is the long-lasting reduction in serum cholesterol levels to more normal values recorded by Mollison and Rennie. These authors also used preparations of dextran of various molecular sizes, and noted more persistent effects with the larger molecular sizes, but greater immediate increases in plasma volume with the smaller—effects which would have been predicted from considerations outlined in previous chapters. The important contributions of Wallenius (1954) in noting the passage into nephrotic urine of larger dextran molecules than are excreted by normal kidneys will be considered in detail in Chapter VI.

REACTIONS FOLLOWING ADMINISTRATION OF DEXTRAN TO MAN

The intravenous injection of any solution may be followed by a febrile reaction unless certain well-known precautions are taken during the preparation of the solution and unless the substances in solution can themselves be prepared free from pyrogens. Experience has shown that dextran solutions can be prepared so that the incidence of febrile reactions is no greater, and in fact often very much less than, the incidence observed after the administration of blood or plasma or saline (Bohmansson *et al.*, 1946; Bohmansson *et al.*, 1948; Thorsen, 1948; Bull *et al.*, 1949; Wilkinson, 1951; Maycock, 1952; Wilson *et al.*, 1952).

Although it was perhaps to be expected that examples of sensitivity to dextran, a bacterial polysaccharide, would come to light, and that this type of reaction might be more troublesome than the febrile reaction, the earlier reports of the use of dextran suggested that such reactions were few. Thus Bohmansson *et al.* (1946) state that sensitivity to Swedish dextran was observed in "occasional cases", and manifested by transient urticaria and pruritus. Bohmansson *et al.* (1948) mention that "unfavourable reactions in other respects", i.e. other than pyrogenic, occurred in less than 1 per cent of 1500 patients, receiving between them 5000 bottles of Swedish dextran. Thorsen (1948), discussing the same series of cases, refers to asthmatic attacks, urticaria, falls of blood pressure and lumbar pain.

Lundy *et al.* (1948), Turner *et al.* (1949) and Lundy *et al.* (1950) reported urticarial and allergic reactions in a high proportion of unanaesthetized patients who were given a solution of Swedish dextran, prepared in the United States from the imported dried

polysaccharide. In the papers of Lundy and collaborators, however, it was also observed that imported Swedish dextran solutions did not cause urticarial or allergic reactions in anaesthetized or unanaesthetized patients.

In a series of 1647 patients, who received a British dextran solution, 15 patients exhibited reactions of an urticarial, allergic or anaphylactic nature (Maycock, 1952). Of these 15 patients, 9 exhibited mild generalized urticaria, one developed marked oedema of the eyelids, and one vomited and complained of persistent headache for a week after infusion. Four patients exhibited more severe reactions, starting shortly after the beginning of the infusion, all of which were characterized by a vasomotor collapse, profuse sweating, coughing, vomiting and cyanosis. In addition the following symptoms or signs were also noted in one or more of these 4 patients; intense hyperaemia of the face and arms, urinary and faecal incontinence, muscular spasms, pain in the loins, pain in the chest, oedema of the face, eyelids and hands. The 15 patients received between them seven different batches of dextran, bottles from all of which had been given to many other patients without untoward effects of any kind.

While this survey of patients was being made in England, it was observed in America that 33 out of 64 unanaesthetized normal convalescent soldiers exhibited reactions to the intravenous injection of 500 ml. of Swedish dextran, that only 4 out of 45 soldiers under spinal or general anaesthesia reacted and that the reactions were milder; and that only 8 reactions occurred among 97 soldiers (of whom 27 were anaesthetized and 2 exhibited reactions) after the injection of 500 ml. of American dextran (Tarrow & Pulaski, 1953). British dextran given under similar conditions to a group of 9 unanaesthetized soldiers was associated with reactions in 4. The symptoms and signs characterizing these reactions were similar to those reported by Maycock (1952), and were ameliorated by antihistaminic drugs.

On the other hand it was known that many thousands of bottles of Swedish and British dextran solution had been given without untoward effect to civilian and military patients suffering from oligaemic shock and other conditions in Europe, and that smaller amounts of these dextran solutions had also been used successfully in America in the treatment of such patients. The fact that

Pulaski's observations were made almost exclusively on a convalescent military population suggested that the high reaction rate might be related to the "immunization state" of the volunteers. However, no significant difference was observed in the incidence of adverse systemic reactions to two different batches of Swedish dextran among 30 immunized and 51 unimmunized soldiers. The overall reaction rate was 51 per cent (National Research Council, January 1952). In this trial no prognostically helpful correlation was found between the local reaction to the intracutaneous injection of clinical dextran solutions and the subsequent development of systemic reactions; a correlation however appeared to exist between the cutaneous reaction to certain more highly branched native dextrans and subsequent reactions. In a later trial (National Research Council, April 1952) a significant degree of correlation was observed in normal volunteers between cutaneous reactions to Swedish clinical dextran and the incidence of systemic reactions following intravenous injections of this dextran.

Heisö & Lund (1953) and Wilkinson & Storey (1953) have also described the occurrence of similar systemic reactions in smaller series of normal unanaesthetized subjects after the infusion of 500 ml. or 1000 ml. amounts of various English, Norwegian and Swedish dextran solutions. Wilkinson & Storey observed in their series of 5 volunteers, all of whom exhibited reactions, that the plasma volume (as followed by haematocrit changes) decreased for some hours after the end of the infusion and then rose to a level above that of the pre-infusion plasma volume. Heistö and Lund, in a series of 29 volunteers, noted that this phase of a reduced plasma volume did not occur in non-reactors. These authors observed in addition that the severity of reactions appeared to be inversely related to the time which had elapsed since T. A.B. inoculation.

It may be concluded that certain individuals appear to be sensitive to dextran, that this sensitivity is manifested by urticaria or a generalized reaction of an allergic or anaphylactic nature, and that evidence of sensitivity is more readily shown by unanaesthetized normal individuals, as opposed to anaesthetized normal individuals, and, apparently, patients whether anaesthetized or not, who are suffering from oligaemic shock or other illness. It also seems clear that these types of reaction are more often

associated with dextrans prepared from certain strains of leuconostoc (Heistö & Lund, 1953; Tarrow & Pulaski, 1953). In the American trials referred to above, the more highly branched dextrans were associated with a greater incidence of reactions than the one less branched dextran used. The results also suggested that dextrans of high average molecular weight were more prone to cause reactions than dextrans of low average molecular weights; confirmatory evidence of this is required.

The overall incidence of sensitized individuals is not known. Except in general terms, the incidence of sensitivity to any one form of dextran is not known; e.g. individuals in the American population who are sensitive to the dextran originally prepared in Sweden and to one of the British types of dextran appear to be more numerous than individuals sensitive to American dextran.

Several alternative explanations for these reactions have been suggested; none has yet been confirmed. Heire & Sugg (1950) and Heire *et al.* (1952) suggested, on the basis of the cross-reactions they had shown between rabbit pneumococcal antisera, types II and XX, and native and clinical dextran, that the antibody-combining properties of dextran should be regarded as a theoretical source of danger in persons who possessed a high titre of the appropriate antibodies at the time of injection. Likewise antibodies to *S. typhi* and other members of the *Salmonella* group (Zozaya, 1932*b*; Neill & Abrahams 1951), or to *Streptococcus, Group H*, grown in sucrose broth (Sugg *et al.*, 1942) all of which have been shown experimentally to cross-react with dextran may be responsible for reactions in humans, although the results of tests on American soldiers appear to exculpate antibodies to *Salmonella typhi* and *S. paratyphosi A* and *S. paratyphosi B*. Unfortunately the titres of antibodies to these various bacteria do not appear to have been investigated in patients exhibiting sensitivity. Secondly, individuals who exhibit sensitivity to dextran, may have become immunized to dextran in one of the ways discussed on p. 43 and produced specific antibodies to dextran. Here again the titre of such antibodies has not been investigated in patients exhibiting sensitivity.

The significance, with regard to reactions, of the presence of agglutinating antibodies to certain strains of *L. mesenteroides* in normal human sera (Warren *et al.*, 1952) is not yet known

A satisfactory explanation of the high incidence of reactions to certain dextrans shown by unanaesthetized normal individuals compared with the low incidence of reactions to the same dextrans shown by unanaesthetized hospital patients has not yet been found. It is possible there may be adrenal cortical over-activity in the latter group, which diminishes, or entirely inhibits, reactions to dextran in individuals who would, under normal circumstances, exhibit sensitivity.

Chapter VI

EXPERIMENTAL USES OF DEXTRAN

IN the preceding chapters, an outline has been given of the chemical properties of dextran so far as they are yet known, and of the behaviour of dextran in the body, both under circumstances of deliberate test and of therapeutic usage. An attempt has been made to indicate the variable effects of dextran preparations differing in molecular size and structure, and arising out of this knowledge, to define the kind of specification needed to ensure the provision of dextran satisfactory as a plasma substitute. So far a number of experimental studies have not been described. Though at first sight some of these might be classed as "academic" they are in fact highly relevant in two distinct ways to the problem of how best to use therapeutic substances like dextran in medicine. In the first place, they indicate in more detail the possible variations of response to different sorts of dextran. Then they throw fresh light on the body's reactions to the administration of foreign macromolecular substances like dextran, and so indirectly on the effects of other natural large molecules. Some investigations likely to be useful were outlined by Squire (1951). Certain limitations of a practical nature have so far been imposed on such studies mainly from the difficulty of supplying suitable preparations of dextran. For clinical purposes, a constant type of preparation is mainly required, and a moderate degree of dispersion in molecular size is tolerable. In general, the experimentalist desires to compare and contrast the effects of different molecular sizes and structures, but would like each preparation to be as homogeneous as possible. Economic considerations, as already mentioned, limit the production of such materials, though several manufacturers have on occasion produced narrow-cut fractions of various sizes for individual tests.

Dextran has naturally been used by the experimental physiologist for the resuscitation of animals and to study homeostatic mechanisms (van den Heuvel, 1949). But the main experimental uses of dextran so far exploited concern its effect on the erythrocyte,

its passage through body membranes, and the development of charged derivatives of dextran.

EFFECTS ON ERYTHROCYTES

The effect of dextran in causing increased rouleaux formation and so raising the erythrocyte sedimentation rate has been described in Chapter III. This effect seems directly analogous to that of fibrinogen (Hardwicke & Squire, 1952) and so can be used to elucidate more fully the mechanism of the rise in erythrocyte sedimentation rate familiar in various disease states. It can also be used to replace fibrinogen for processing of blood into various fractions; admixture of say one part of 6 per cent dextran (preferably with molecular weight 200,000—500,000) with ten parts of whole blood causes rapid aggregation and sedimentation of erythrocytes, leaving leucocytes, platelets and plasma proteins in the supernatant layers in a state suitable for further processing as required. The details of the physicochemical process by which dextran causes erythrocyte aggregation are still unknown. Attempts to show that dextran is adsorbed on to the erythrocyte surface (Ryttinger *et al.*, 1952) have not been successful with the chemical methods so far used, and with the more delicate serological method, Maycock & Lorenz (unpublished observations) have shown that the red cells can readily be washed free from dextran. In this and other experimental applications the use of radioactive dextran synthesized from ^{14}C-containing sucrose may give valuable results.

Attempts to use dextran in place of albumin as a medium for detecting incomplete rhesus antibodies, first suggested by Grubb (1949), have been only moderately successful. Richardson-Jones (1950) showed that the dextran concentration was critical since there was only a small range within which it gave reliable results; high concentrations led to rouleaux formation. Ikin (see Mollison *et al.*, 1952) showed that small molecular weight dextrans (22 000 and 38,000) failed to give good agglutination; dextrans of larger molecular weights (124,000, 220,000 and 700,000) caused incomplete anti-D antibody to agglutinate D-positive cells to a high titre. The agglutination effect appeared to increase with molecular weight, but for any given dextran there was only a narrow effective range of concentration; above this range false positive reactions due to rouleaux were observed and below it satisfactory agglutina-

tion did not occur. Dextran cannot be recommended for routine use in blood grouping in place of albumin, since each preparation of dextran solution would have to be standardized within narrow limits before it could be used. When "narrow-cut" fractions of dextran are available they may find a place in the blood grouping laboratory.

PASSAGE OF DEXTRAN THROUGH NATURAL MEMBRANES

In clinical use, the colloid osmotic effect of dextran depends on its retention within the vascular system for a sufficient period. This restriction of the passage of dextran through a membrane freely permeable to crystalloid substances, such as the capillary walls in various parts of the body, can be imitated *in vitro* by suitable grades of nitrocellulose membrane. Such membranes are required for the measurement of colloid osmotic pressure with dextran solutions (cf. Chapter II). They have also been used in the laboratory for concentration of weak solutions of protein, such as the cerebrospinal fluid; specimens of cerebrospinal fluid are placed in a small nitrocellulose thimble which is then dipped in a strong solution (e.g. 20 per cent) of dextran. Within a few hours, much of the fluid has been withdrawn by osmotic forces through the thimble membrane, leaving behind a concentrate of cerebrospinal fluid suitable, for example, for further examination by the technique of paper electrophoresis.

Whereas nitrocellulose membranes of small mean pore diameter retain both plasma proteins and dextran, others constructed to have larger pore sizes can be used in ultrafiltration experiments to separate at any rate partially the smaller molecules of plasma from the larger (e.g. Grabar, 1938). An apparently similar process occurs in the kidney as suggested by the finding that dextran molecules excreted in the urine are of smaller *average* molecular size than those of the polydisperse preparation infused (Bull *et al.*, 1949). As alternative explanations of this finding could be advanced (e.g. breakdown of large molecules to small within the body), Brewer (1951) carried out deliberate tests in rabbits using narrow-cut fractions of molecular weight 5,000—10,000, 25,000 and 38,000. By measuring the renal clearance of these preparations at various levels of plasma concentration, Brewer was able to show that the renal tubules did not appreciably affect excretion either by secretion or by reabsorption. The problem therefore concerns

the permeability of the renal glomeruli to macromolecules. In the rabbit, at least, creatinine clearance is regarded as a satisfactory measure of glomerular filtration rate for water and other small molecules. By comparison of dextran clearance with creatinine clearance, it was found that whereas the low molecular weight (5,000—10,000) dextran passed the glomerular membrane as readily as water, those of molecular weights 25,000 and 38,000 only passed at 19 per cent and 6 per cent respectively of the rate of passage of water. This finding represents a fundamental step forward in our understanding of glomerular filtration, since the previous classical concept based on the excretion of haemoglobin and retention of albumin suggested a sharp differentiation between molecules just below and above the molecular weight range 64,000—68,000 (Bayliss *et al.*, 1933). Translated into a membrane pore theory—the simplest way of interpreting these new results—all of the pores in the glomerular membrane transmit molecules up to about 10,000 molecular weight, and, whereas some of the pores are unable to transmit larger molecules, others can. Some form of "normal" distribution of pore size is indicated by these results and would indeed be in line with general biological principles. These findings accord with the graded permeability to protein molecules of various sizes of the kidney in the nephrotic syndrome (Squire, 1953).

The "renal clearance of dextran as a measure of glomerular permeability" has been very fully examined by Wallenius (1954) (cf. p. 24). Wallenius has carried out numerous tests in dogs and in humans with normal or protein-leaking kidneys. Detailed characterization of the dextran fractions used is a feature of this work. These studies in dogs and in normal humans are much more extensive than those of Brewer, and fully confirm that work. A most important finding is that the glomeruli of those patients with proteinuria transmitted larger molecules of dextran than did normals. A very careful discussion is given of the general relationship between the size of the penetrating molecule and the structure of the membrane. Here Wallenius considers the theory of disperse pore size, pointing out that differential rates of filtration could occur to some extent even through a membrane with homogeneous pores if these were only just large enough to transmit the macromolecules concerned (i.e. would exert a kind of frictional slowing of macromolecules

relative to water and other small molecules). He also refers to the ideas of Chinard (1952) who, like Govaerts & Lambert (1953), considers that diffusion of macromolecules must be considered as well as ultrafiltration.

So far, the renal glomerulus is the only body membrane adequately studied in this way. It is to be hoped that such work will stimulate consideration of other special sites such as the blood cerebrospinal-fluid barrier, the placenta and the membrane separating the interior of joints from the circulation. As with the kidney, valuable information might be forthcoming of alterations in disease, for certainly there is frequently to be found increased transmission of the larger plasma protein molecules in inflammatory states. Little is yet known of the transmission of dextran by the general capillary bed, though Wallenius (1950) has argued from indirect evidence that small dextran molecules pass into the oedema fluid of nephrotic patients. Claims have also been put forward that a plasma substitute of larger than normal average molecular size is better retained within the circulation (Boyd *et al.*, 1953), but further work with narrow-cut fractions is required properly to evaluate this suggestion.

CHARGED DERIVATIVES OF DEXTRAN

So far in this book, simple dextran degraded by hydrolysis has been considered. This is a substantially uncharged molecule, and therefore relatively inert in its chemical behaviour, in or out of the body. But by treatment of partially hydrolyzed dextran with chlorosulphonic acid in the presence of pyridine, dextran sulphates are produced, and these highly charged molecules have an entirely different series of reactions. The main use of dextran sulphate so far has been as an anticoagulant having properties in this and certain other respects similar to those of the endogenous product, heparin (Ricketts & Walton 1953). Lest any confusion arise it should again be stressed that dextran itself has no appreciable anticoagulant effect *in vitro* or *in vivo*.

A full discussion of the properties of the dextran sulphates, which depend not only on the molecular size but on the degree of sulphation (i.e. charge) would occupy another monograph. But the principle that new properties are conferred by the introduction of electrical charge into a macromolecule is worth consideration in the general context of plasma substitutes. Plasma itself con-

sists of a mixture of at least 12 main protein components, the specific functions of which are becoming increasingly understood. So far the plasma substitutes proposed for practical use consist of macromolecules similar in size to serum albumin; they possess the single useful quality, so far as is known, of colloid osmotic pressure so that their administration can tide over a period during which the circulating plasma protein mass is depleted until the body can once more achieve a balance between production and utilization of the various plasma fractions. The inertness of the substances chosen, especially marked in the case of dextran, is no doubt responsible broadly for the impunity with which, in moderate dosage at least, they can be introduced. But if these macromolecules can now be further modified by chemical treatment, is it not possible to provide solutions (probably mixtures of various chosen fractions) which would subserve other vital functions of the plasma? Transport of metals such as iron and copper (naturally a function of β-globulins), of bile pigments (a function of serum albumin) and of lipids (functions of α- and β-globulins) are obvious examples of properties which might usefully be imitated. Charge, too, may be of importance in restricting the passage even of macromolecules through membranes. At physiological pH, the plasma proteins, though still acting as buffers, are predominantly behaving as weak bases. Hints such as these at least suggest further search for plasma substitutes which might successfully replace rather more than one-half of the plasma—the level beyond which evidence suggests currently available materials cannot be given without some risk of harm. Too much success should not, perhaps, be anticipated—at present it is hard to see how some of the very specialised functions of plasma could in any way be imitated. But with the initial work of Bayliss and others on gum arabic still less than 40 years old, while electrophoretic and ultracentrifugal analysis of the composition of plasma is even more recent, the possibilities of more marked progress are surely still very great.

APPENDIX I

NOTES ON SOME USEFUL LABORATORY METHODS

(1) PRESERVATION OF SOLUTIONS

Moulds grow readily in dextran solutions. Sodium azide in a final concentration of 0.01 per cent w/v has been found useful in preserving solutions for physical measurements. For physiological work sterilisation by autoclaving at a pressure of 15 lbs. per sq. in. for 20 minutes is effective. The solution must be neutral otherwise partial hydrolysis with change in molecular weight distribution will occur. If reducing sugars are present some browning of the solution occurs.

(2) DEXTRAN IN POWDER FORM

Clinical dextran is dialyzed to remove salt if necessary and concentrated under reduced pressure to 10—20 per cent dextran. The concentrated solution is run slowly from a tap funnel into a rotating blade mixer containing about 10 volumes of absolute alcohol when a powdery precipitate should be obtained. Too much water makes the product sticky. The alcohol is then decanted and after mixing with fresh alcohol the suspended powder is centrifuged, washed with ether and dried *in vacuo* over phosphoric oxide.

Alternatively a fraction may be precipitated from clinical dextran by gradual addition of acetone and standing at constant temperature. The syrup so precipitated is then run into alcohol, as described. Too much acetone makes the syrup too viscous to pour.

Attempts to add sufficient precipitant to cause complete precipitation from 6 per cent solution lead to a sticky product which may take weeks to harden to a powder in alcohol. Complete recovery is therefore best accomplished by freeze-drying.

(3) MEASUREMENT OF CONCENTRATION

Standard. A pure dextran preparation is essential to standardize all methods. Samples of clinical dextran after dialysis are usually sufficiently pure for this purpose. About 100 mg. pure dextran in 2 ml. water is frozen on the walls of a 10 ml. ampoule and the water removed by sublimation at 0.1 mm. mercury pressure. Drying is continued at 100°C. and less than 0.1 mm. Hg over phosphoric oxide to constant weight. Using pure dry dextran for preparation of standard solutions, accurate concentrations can be calculated from:

(*a*) optical rotation: (*b*) refractive index: (*c*) chemical methods.

(*a*) Optical rotation, $[\alpha]_D^{20} = \frac{100\theta}{lc}$ where θ is the observed rotation

in sodium "D" light at 20°C., c is concentration in g. per 100 ml. and l is length of solution in decimeters. For NRRL—B512 clinical dextran $[\alpha]_D^{20} = 195° - 200°$.

(*b*) Refractive index. For pure water the refractive index at 20°C. is 1.3330 and the specific refractive increment for dextran is 0.00153 per g. per 100 ml. in light of wavelength 436 $m\mu$.

(*c*) Chemical determinations. The principles involved in various methods are described on page 20 *et seq.* For most purposes satisfactory results may be obtained with the anthrone reagent (Roe, 1954) or a similar but stable reagent (Lunt & Sutcliffe, 1953).

(4) INTRINSIC VISCOSITY

Four solutions with accurately known concentrations of about 2, 1.5, 1.0 and 0.5 g. per 100 ml. in water (or saline) are prepared. The relative viscosity of each solution is measured using a U-tube viscometer with flow time for water of about 100 seconds. Specific viscosity is obtained by subtracting 1.00 from the relative viscosity. Specific viscosity divided by concentration is plotted against concentration, giving a line which can be extrapolated to the viscosity axis, the intercept being the intrinsic viscosity $[\eta]$. For very precise work a correction for the kinetic energy of the moving solution in the viscometer may be introduced and measurements may be needed on more dilute solutions.

APPENDIX II

Summary of Possible Variations of Clinico-Pathological Findings in Patients who have received Dextran

BLOOD

Erythrocyte sedimentation rate.	Raised (see p. 23).
Haematocrit, haemoglobin, red and white cell counts.	Lowered in proportion to dilution by dextran for varying periods.
Histology and relative numbers of formed elements.	Unchanged.
Copper sulphate method for plasma protein.	With high dextran levels drops may not form. Value obtained is an index of total colloid and not necessarily of plasma protein.
Paper electrophoresis of plasma protein.	Pattern not affected.
Blood sugar estimation.	When alkaline copper reagents are used some of the copper may form a complex with dextran as in Hint & Thorsen method of estimation of dextran (see p. 21).
Blood grouping and crossmatching.	Some interference at high dextran levels due to rouleaux formation (see p. 30).

URINE

Specic gravity.	Raised during excretion of dextran.
Glucose.	As for blood sugar (see above.)

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FAILURE OF LOW MOLECULAR WEIGHT DEXTRANS TO ALTER THE FREQUENCY OF LUNG METASTASIS

Report on the V2 Carcinoma of the Rabbit

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Three fractions of low molecular weight dextran (10,000, 40,000 and 70,000) were administered intravenously to Laboratory Lop rabbits before or after intravenous inoculation of ascitic V2 carcinoma cells. The frequency of pulmonary metastases was unaltered by dextran treatment. No extrapulmonary tumor was found in any instance. These findings are discussed in relation to the studies of other investigators and the need for additional experiments employing a variety of tumors and hosts is emphasized.

THIS REPORT RECORDS THE FAILURE OF LOW molecular weight dextrans (M.W. 10,000, 40,000 and 70,000) to influence the frequency of pulmonary metastases resulting from the intravenous inoculation of ascitic V2 carcinoma cells in rabbits.

Low molecular weight dextran has been reported to coat the erythrocytes, platelets and vascular endothelium[6] and has been used as a "desludging" or antithrombotic agent in a variety of experimental and clinical situations.[7] Most of these experiments have been performed with traumatically induced venous thrombi. Such thrombi are initially and predominantly composed of platelets,[15] contain little or no fibrin[15-19] and may form in the absence of fibrinogen or fibrin.[18] It has been suggested that the function of dextran may be related to its action on platelets. Gurewich and Fasanaro[17] have demonstrated that dextran is ineffective in preventing atraumatic venous thrombosis whereas heparin is effective.

The mechanisms of metastasis formation from blood-borne tumor cells have been reviewed previously.[13, 31, 35] By direct in vivo microscopy, Johnson and Wood[21] described the sequence of events occurring when a suspension of ascitic V2 carcinoma cells is infused intravascularly by microcannulation of small arteries or arterioles (40 to 100 μ). Three separate types of thrombi are formed:

1. A firm *injury thrombus* (or hemostatic plug), composed predominately of platelets, forms almost instantaneously about the puncture wound in the vessel wall;

2. When V2 carcinoma cells were slowly infused, they were enmeshed within a tenacious fibrin matrix with remarkable speed; *tumor thrombi* are softer and more delicate than those of the *injury type* and both are devoid of erythrocytes;

3. Fifteen to 20 min after formation of the tumor thrombus, a friable and delicate *red cell thrombus* occurs by aggregation of erythrocytes.

The ultrastructural details of these thrombi await evaluation. Selected lots of urokinase activated or streptokinase activated plasminogen (plasmin) will lyse the red cell and tumor thrombi but produce no alteration of injury thrombi. When infused *after* the intravenous injection of cancer cells, these fibrinolytic agents significantly reduced the frequency of metastatic tumor.[35] Protection against experimental metastatic tumor has been reported with both anticoagulants and fibrinolytic agents.[2, 8, 10, 12, 34, 35]

Cancer cells, especially those of the V2 car-

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cinoma, are rich in thromboplastic activity. Lawrence et al.[23, 24] assayed suspensions of cells derived from the solid form of this tumor in rabbits and found a correlation between in vitro shortening of the prothrombin time after intravenous injection and in vivo thromboplastic activity. Thornes[33] noted that extracts of the solid form of the V2 carcinoma contain 8 to 10 times the thromboplastic activity of the ascitic form and that the activities of both were predominantly heat stable. Holyoke and Ichihashi[20] reported that the transplanted mouse sarcoma T241 and spontaneous mammary tumor (C_3H) possess both heat stable and heat labile thromboplastic activity. In contrast, breast tissue from precancerous, force-bred females shows little extractable thromboplastin activity. O'Meara, Thornes and associates[6, 20, 27, 33] have described cancer coagulative factors (CCF) in human tumors. The thromboplastic activity of CCF is inhibited by fibrinolytic agents[34] or low molecular weight dextran (10,000).[33]

The effects of low molecular weight dextran (10,000) on intravascular tumor thrombi were observed using in vivo microscopy.[21] In 5 separate experiments, there was no inhibition of thrombus formation. No lysis of tumor, injury or red cell thrombi occurred. Despite these negative findings, the persistence of an interest in the possible use of low molecular weight dextrans (10,000, 10,000 or 70,000) to prevent or disrupt aggregation or endothelial adhesion of blood-borne cancer cells led to the additional experiments reported below.

MATERIALS AND METHODS

Laboratory Lop rabbits* weighing 1.3 to 2.8 kg were caged individually and were provided with Purina lab chow and water ad libitum throughout each experiment. The transplantable tumor employed was the ascitic form of the V2 carcinoma containing 5-10 × 10^5 cells/ml, harvested under sterile conditions. A 2 ml aliquot was diluted 1:500 with warm (37.5°C) Morgan and Parker's Mixture 199 (containing bicarbonate buffer, penicillin and chloramphenicol) in a large beaker and was agitated gently with a magnetic bar and rotator. All injections were performed intravenously via a 22 G needle inserted into the marginal vein of the ear. Each rabbit received a single inoculation of 10 ml of diluted ascitic fluid containing 25-50 × 10^3 cells.

Four separate experiments are reported. In each the dextran solutions, 5% dextrose in water and normal saline, were administered intravenously.

Experiment I: Forty-five rabbits were divided randomly into 3 equal groups averaging 2.0 kg.

Group 1 animals (controls) received 5.2 ml/kg of 5% dextrose in water immediately before inoculation of tumor. Low molecular weight dextran [average molecular weight 10,000, 10% solution in 5% dextrose in water, Pharmacia lot no. T0644 (dextran-10)] was administered in a dose of 15 ml/kg (1.5% of body weight) immediately *prior* to injection of tumor cells in group 2 and *immediately following* tumor cell injection in group 3.

Experiment II: Thirty rabbits were randomized into 2 groups averaging 1.7 kg. Five doses of low molecular weight dextran [Rheomacrodex,† average molecular weight 40,000, 6% solution in normal saline, Cutter lot no. TT73241B (dextran-40)] were administered.

After inoculation of tumor cells, the controls, group 1, received 5.2 ml/kg 5% dextrose in water at one-half, one, 2, 3 and 4-hour intervals. Group 2 received tumor cells followed by 14.5 ml/kg of dextran-40 at similar intervals, a total of 72.5 ml/kg (7.25% of the body weight).

Experiment III: Forty-five rabbits were divided into 3 groups, each containing 15 animals and averaging 2.3 kg. Group 1 control animals received 5% dextrose in water, 13 ml/kg at 60 min and 6.5 ml/kg at 90 min, following the inoculation of tumor cells. Low molecular weight dextran [clinical dextran, average molecular weight 70,000, 5% in normal saline, Cutter lot no. TT3293B (dextran-70)] was administered to group 2 in a dose of 21.7 ml/kg and 10.8 ml/kg 60 and 90 min after injection of tumor. A total of 32.5 ml/kg of dextran-70 was inoculated, an average of 75 ml/rabbit or 3.25% of the body weight. Group 3 received the same doses of dextran-40 at 60 and 90-min intervals after tumor.

Experiment IV: Thirty rabbits were divided into 2 groups averaging 2.3 kg. Twenty-five min after inoculation of tumor, group 1 controls received 15.2 ml/kg of normal saline. In group 2 animals dextran-70 (Cutter lot no. LTT8293A), 15.2 ml/kg, (1.5% of the body weight) was injected 25 min following tumor.

All rabits were killed 25 to 29 days after injection of the cell suspension and the amount

* Bred at the Bar F Rabbitry, Perry Hall, Md.
† Pharmacia Laboratories, New Market, N.J.

and location of tumor were recorded without knowledge of the treatment received until all the animals in an experiment were necropsied. The number of lung tumors in each rabbit were recorded by one individual (S.W.). No extrapulmonary tumor was found.

RESULTS

The results of these 4 experiments are presented in Table 1. Dextran-10 administered in a dosage of 15 ml/kg (1.5% of the body weight) gave no protection when administered immediately before or after the tumor. Dextran-40 in 5 divided doses during a 4-hour period (total dosage 7.25% of the body weight) provided a slight but significant protective effect ($P<0.05$). However, in experiment III dextran-40 and dextran-70 administered at 60 and 90-min intervals following the injection of tumor in a dosage equal to 3.3% of the body weight proved ineffective. When dextran-70 was injected 25 min following the tumor in a dosage equal to 1.5% of the body weight (experiment IV), there was no significant protection.

TABLE 1. Effect of Dextran on Lung Metastasis

Group	No. of rabbits	Dose ml/kg	Mean no. lung tumors ± SE*
Experiment I			
Control (5% dextrose in water)	13	15.0	94.62± 9.10
Dextran-10 (pretreatment)	11	15.0	83.86± 7.91
Dextran-10 (post-treatment)	12	15.0	91.33±10.77
Experiment II†			
Control (5% dextrose in water)	13	26.0	58.80± 3.86
Dextran-40 (post-treatment)	15	72.5	44.71± 4.29
Experiment III			
Control (5% dextrose in water)	13	19.5	11.29± 1.55
Dextran-70 (post-treatment)	14	32.5	12.60± 1.69
Dextran-40 (post-treatment)	14	32.5	11.93± 1.99
Experiment IV			
Control (saline)	14	15.2	67.71± 4.87
Dextran-70 (post-treatment)	14	15.2	55.86± 6.17

* Standard error.
† In this experiment the level of statistical significance for the control vs. treated group revealed $P < 0.05$.

DISCUSSION

Various fractions of low molecular weight dextran have been evaluated in a number of experimental tumor systems. Griffin and Aust[16] have demonstrated that dextran-10 is capable of reducing metastatic tumor deposits when given prior to the injection of tumor cells in the mouse; however, these authors also found that, when metastases occur in the dextran pretreated group, they were of greater number and size.

In an extensive series of experiments with rats utilizing the intraportal injection of Walker 256 carcinosarcoma cells, Fisher and Fisher found that the number of hepatic metastases was increased by dextran treatment. This increase appeared to be related to the expansion of circulating blood volume resulting from the dextran infusion; however, another factor may have played a role in these experiments. In the rat, a species-specific reaction to dextran has been described. Dextran generally causes increased capillary permeability with edema.[22, 28] Most authors relate this effect to histamine liberation but there are also some indications that other mechanisms may be involved (e.g., anaphylactoid reactions of the immediate hypersensitivity type).[1-4] Such adverse reactions in this species may account for the increased incidence of hepatic metastasis in dextran-treated rats.

Alexander and Altemeier[3] were unable to alter the frequency of metastases from blood borne V2 carcinoma cells developing in surgical incisions of dextran-70-treated rabbits. Schatten et al.[30] reported that dextran-70 significantly decreased the number of V2 carcinoma metastases in the lungs of New Zealand white rabbits. Suspensions of tumor cells were prepared from small pieces of solid tumor passed through a cytosieve. The suspensions were diluted with saline to a concentration of 1×10^5 cells/ml and each pentobarbital and ether-anesthetized animal received one ml injected into a surgically exposed femoral vein. When administered either 30 min prior to or 30 min after the injection of tumor, dextran-70 (2% of the body weight) reduced the number of gross pulmonary metastases by 89 and 66%, respectively. While our experiments and those of Schatten were performed in the same species of animals with similar experimental tumors, there were differences in the strains of rabbits used, the form of tumor cell suspensions, anesthesia, position of the animals and extraordinarily high frequency of hepatic tu-

mors. In our experiments the number of tumor cells infused was greater than that reported by Schatten; however, fewer pulmonary tumors developed in our control animals than in those of Schatten. The difference in results may be attributable to the use of an ascitic form of tumor in our experiments versus a thromboplastin-rich solid tumor in Schatten's experiments.

The clinical status of dextran-40 recently has been reviewed critically by Couch.[11] He emphasized the lack of proof that the most important clinical effect of dextran-40 was related to factors other than plasma expansion and dilution of erythrocytes, fibrinogen and globulin. In in vitro studies Meiselman[25] analyzed the so-called "flow-improving" properties of dextran 40 on fresh, anticoagulant acid citrate dextrose human blood. These data indicated that only by hemodilution, with the accompanying reduction in hematocrit, was it possible to reduce the viscosity of whole blood. No evidence of a reduction of erythrocyte interaction (or aggregation) was found. Similar findings have been reported from in vivo studies in dogs by Replogle et al.[26] who concluded that dextran-40 *without* hemodilution produced no changes in blood flow. These data are in agreement with the direct in vivo microscopic observations herein reported that dextran-40 was ineffective in altering tumor cell–cancer cell aggregation or tumor cell–endothelial cell adhesions or metastatic implantation.

Dextrans of varying molecular weights have been effective in preventing metastasis in some studies and completely ineffective or harmful in others. These differences in results indicate the need for further experimental evaluation prior to a clinical trial of dextran in the prevention of tumor implantation and growth.

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